JUN 5 1925

Medical Lib.

BULLETIN

OF THE

JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

Vol. XXXVI, No. 3 MARCH, 1925

Contents

The Epithelioid Cells of Tuberculous Lesions. M. R. Lewis, H. S. Willis and W. H. Lewis..... 175

Evidence Showing the Existence of Two Distinct Types of Pigment Cells Capable of Giving Rise to Melanotic Tumors. David T. Smith..... 185

Report of the Recovery of T. Pallidum from the Spinal Fluid of a Patient with Syphilitic Meningitis of the Neuro-Recurrence Type. Jarold E. Kemp and Alan M. Chesney..... 199

Hyperplasia of Lipoid-Holding Cells in Diabetes with Lipemia. Margaret G. Smith..... 203

The Fate of the Sutures as Observed in Intestinal Anastomosis and in the Healing of Wounds. Frederick Leet Reichert and Emile Holman..... 212

Book Reviews..... 222

PUBLISHED MONTHLY

THE WILLIAMS & WILKINS COMPANY

BALTIMORE, U. S. A.

Made in United States of America

THE BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

EDITORIAL BOARD

Edward P. Carter — Arnold R. Rich
Wilburt C. Davison — Frank R. Smith
George B. Wislocki

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of the Johns Hopkins Hospital, Baltimore, Md.

Books for review should be sent to The Editor at the above address.

Fifty reprints, with covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

Correspondence concerning business matters should be sent to The Williams & Wilkins Company, Publishers of Scientific Journals and Books, Mount Royal and Guilford Avenues, Baltimore, Maryland, U. S. A.

This periodical is issued monthly. The current volume is 36.

Subscription is by the volume only, and not by the year. Two volumes a year are issued, each consisting of approximately 400 pages.

Subscription price per volume, postpaid. In the United States and all countries that are members of the Postal Union, $3.00. In countries that are not members of the Postal Union, $3.50. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

PUBLISHERS' AGENTS

For Argentina and Uruguay: Beutelspacher y cia, Sarmiento 815, Buenos Aires.

For Australia: Stirling & Co., 317 Collins St., Melbourne; (or Angus & Robertson, 89–95 Castlereagh St., Sydney).

For the British Empire, except Australia and Canada: Send to any British bookseller or forward direct to the Publishers

For Canada: Wm. Dawson & Sons, Ltd., 87 Queen Street East, Toronto.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kjöbenhavn.

For France: Emile Bougault, 48 Rue des Ecoles, Paris.

For Germany: R. Friedländer & Sohn, Buchhandlung, Carlstrasse 11, Berlin.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 11 to 16 Nibonbashi Tori-Sanchome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

For Spain: Ruiz Hermanos, Plaza de Santa Ana, 13, Madrid.

Publishers of
The Bulletin of the Johns Hopkins Hospital
Journal of Comparative Psychology
Journal of Pharmacology and Experimental Therapeutics
Journal of Immunology
Journal of Bacteriology
Abstracts of Bacteriology
Journal of Urology
Annals of Clinical Medicine
Chemical Reviews

THE
WILLIAMS & WILKINS COMPANY
Publishers of Scientific Journals and Books
BALTIMORE, U. S. A.

Publishers of
Comparative Psychology Monographs
Medicine
Journal of Personnel Research
American Journal of Tropical Medicine
Archives of Occupational Therapy
Journal of Dairy Science
Soil Science
Botanical Abstracts
Childhood Education

Entered as Second-Class Matter at the Baltimore, Maryland, Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 3, 1918.

Copyright, 1925, by The Williams and Wilkins Company

The Epithelioid Cells of Tuberculous Lesions

By M. R. Lewis, H. S. Willis, and W. H. Lewis

Baltimore, Maryland

FROM time to time investigators in the field of tuberculosis have returned to the puzzling question of the origin and nature of the epithelioid cell—the essential cell in tubercle formation. However, this question has not as yet received a solution that is universally accepted. The predominating theories of origin—that they come (1) from connective tissue, (2) from endothelium, or (3) from leucocytes—still have ardent supporters. Our present observations are confined to the question of the relation of these cells to the leucocytes; therefore, the merits of the endothelial and of the connective-tissue theories of origin need not be considered here. The claim for the exclusive leucocytic origin dates back to the time of Cohnheim, R. Koch, and Metschnikoff. Since then numerous investigators have claimed that these cells develop, in part at least, either from the lymphocytes, the large mononuclears (endothelial leucocytes), or the clasmatocytes (polyblasts). The recent short review of Maximow (1) obviates the necessity of analysing the literature again.

Recent studies (1), (2), (3) on the transformation of certain of the white blood-cells into macrophages, epithelioid cells, and giant cells of the Langhans type stimulated us to examine more critically the epithelioid cells from tuberculous lungs of the rabbit in fixed specimens, in supravitally stained spreads, and in tissue cultures. We were led further to compare them with the epithelioid cells derived from the white blood-cells in cultures of blood of the rabbit and of other animals, as well as with the clasmatocytes so abundantly present in the normal lung. We first assured ourselves that the living cells which M. R. and W. H. Lewis (2), (3) had, in their blood cultures, designated as epithelioid cells were identical with the living cells abundantly present in the tubercles of fresh spreads of tuberculous lungs of the rabbit. We then compared them with the epithelioid cells in sections of the same material and found that they were of the same type. The living epithelioid cells, as seen in cultures and spreads, bear little resemblance to those seen in sections stained with haematoxylin and eosin, and, if we had depended solely upon such sections, it would not have been possible to see enough resemblance between them and the living ones to assure ourselves of their identity. We were fortunate, how-

ever, in obtaining preparations with a special stain,[1] which did show in part some of the peculiar features of epithelioid cells.

LIVING EPITHELIOID CELLS

The term "epithelioid" is a much used one which was originally applied to the large vesicular, epithelial-like cells in anatomic tubercle and simply implies a cytological similarity to, but not an identity with, epithelium. Later the term has been applied to cells other than those in tubercle and the following description characterizes what we conceive of as a living epithelioid cell based on examination of fresh living tissue spreads of tubercles and tuberculous lungs.

The fully developed epithelioid cells are very conspicuous when stained supravitally with neutral red. They are highly plastic and vary tremendously in size and form, especially in cultures, where they may become highly phagocytic and migratory. They possess a rather large, finely granular central area with a more or less radial arrangement of the granules that have an affinity for neutral red about a central point, presumably the centrosome. Both the central area and the granules composing it vary in size. The larger granules tend to occupy the more peripheral regions. About the central area is a granular zone of fat globules, mitochondria, and possibly other granules. This intermediate zone varies in width in different cells, and the fat globules vary in number and size. The mitochondria, as described by Lewis and Webster (4), lie among the fat globules and may project out into the rather clear peripheral layer of ectoplasm. The nuclei vary from round to elongated curved bodies which lie in the intermediate zone close to the periphery of the central area. The cell, with its outstanding characteristics, is illustrated in Figures 6, 7, 20, and 21. Similar cells, having a large central area with an affinity for neutral red, an intermediate zone of fat globules and mitochondria, and a clear peripheral zone were described and pictured by Lewis and Webster (4) under the names of large wandering cells and endothelial cells. Maximow's (1) Figures 2, 4, 5, 7, and 11, of epithelioid cells, undoubtedly represent the same type of cells that we have called epithelioid cells. He described them as containing a large "homogeneous or faintly granulated attraction sphere", surrounded by a zone containing many bacilli. Our results agree, in part at least, with those of Maximow, if we consider the large mononuclear or the modified large mononuclear as corresponding to the polyblast.

EPITHELIOID CELLS IN FIXED SECTIONS

The pieces for sections and spreads were taken from the lungs of rabbits that had been inoculated in an ear vein fourteen days previously with one cubic centimeter of a heavy emulsion of bovine tubercle bacilli. The lungs were thickly peppered with small tubercles. As already noted, the ordinary haematoxylin and eosin sections afford one a very poor idea of the essential cytological characters of these cells. Sections of the same tissue, however, with our special stain revealed in the looser parts of many of

[1] To be published later when the method is perfected.

the tubercles, more especially in the centers (Fig. 1), numerous large epithelioid cells with a blue central area surrounded by a brownish zone and an eccentrically located nucleus. The majority of the epithelioid cells of the tubercles did not, however, contain such blue centers. Since the area stained blue corresponds to the central area which in the living cells stains with neutral red, it seems probable that the majority of the cells of the tubercles had not developed a sufficient number of granules in the central area for the blue staining to become apparent. As will be seen later, it does not appear to be as delicate a stain for the granules of the central area as the vital neutral red; or it seems to show only when the central area is quite marked. The giant cells within the tubercles were stained in precisely the same manner as the large epithelioid cells.

Much to our surprise, we found many large epithelioid cells and an occasional giant cell of the same type (Figs. 2 to 5) free in the alveoli. These cells contained the characteristic blue central areas and were identical with the cells seen in the tubercles. In some of the cells we were able to see in the center of the central area a small clear centrosome about which the blue granules showed a more or less radial arrangement. Some of the large binucleate cells had two such clear unstained centrosomes. Frozen sections of similar material fixed in formalin and stained with Scharlach R contained clusters of large cells within the tubercles, in which numerous red stained fat globules were arranged in a zone about a central area. The main features of the typical epithelioid cells, as seen in the living, were thus identified, though with some difficulty, in fixed and stained material.

EPITHELIOID CELLS IN SPREADS FROM THE TUBERCULOUS LUNG

An examination of the living cells in spreads from fresh tuberculous lungs with neutral red gives one a very much better idea of the character of epithelioid cells than any sectioned material. Spreads were made by cutting up pieces of the tuberculous lung into small bits, about a millimeter in diameter, in Locke solution both with and without neutral red, and putting two or three on a slide with a fine pipette and dropping on the coverglass. As the lungs were heavily peppered with small tubercles, almost every spread contained one or more of these structures, partly cut across, showing along the cut edge many epithelioid cells and an occasional giant cell. Along such cut edges many large spherical epithelioid cells (Figs. 6 to 8) escaped into the surrounding fluid and floated or rolled about in the currents caused by the slow drying of the preparation. From these free, isolated, living, spherical cells that were stained with neutral red an excellent idea was obtained of certain features of their structure. The large granular central areas which stained with neutral red varied in size. The granules were, as a rule, rather small and, under favorable conditions, were seen to be radially arranged about a clear central point, presumably the centrosome. The larger granules were usually more peripherally located. On the other hand, in unstained preparations the central area, although conspicuous, was pale as contrasted with the region

just about it, which usually contained fat globules. In the spherical cells the intermediate zone, generally free of granules having an affinity for neutral red, occupied most of the region between the central area and the surface of the cell. It was highly granular and contained varying numbers of fat globules, mitochondria, and possibly other granules. Sometimes the fat globules were so fine as to be scarcely recognizable. The oval nuclei were in the intermediate zone, and thus always eccentric, while the centrosome occupied approximately the center of the cell. The cells were covered by a clear, thin, hyalin-like peripheral zone which often extended out into short, irregular, membranous pseudopodia. The diameter of the epithelioid cells was often several times that of the red blood-cells.

The giant cells (Fig. 9) seen in the spreads had precisely the same structure as the epithelioid cells. They were, of course, larger and the nuclei (two to several) were arranged about the central area in the intermediate zone or between the two zones.

In addition to the epithelioid cells, the spreads contained varying numbers of clasmatocytes (macrophages), which were crowded full of vacuoles and granules with an affinity for neutral red; and many intermediate forms, between these and the epithelioid cells, were also noted.

There seemed to be many more cells with the large red central areas in the spreads than cells with blue-stained central areas in the sections. The red-stained central areas are also much more easily seen than the blue-stained ones.

FIG. 1. PHOTOGRAPH OF A SMALL TUBERCLE WITH LARGE WELL-DEVELOPED EPITHELIOID CELLS IN THE CENTER

Many of these cells contain a blue-stained central area which does not show very well in the photograph. From the rabbit's lung, 14 days after intravenous inoculation. × 620 dia.

FIGS. 2, 3, 4, AND 5. EPITHELIOID AND GIANT CELLS FROM THE ALVEOLI OF THE SAME SECTION

They are precisely similar to those within the tubercle, but as they are not so crowded their structure is better seen. × 1350 dia.

FIGS. 6, 7, AND 8. EPITHELIOID CELLS FROM SPREADS OF THE SAME TUBERCULOUS LUNG

The granular central areas were stained with neutral red. The surrounding intermediate zone consists of fine fat globules and mitochondria. × 1350 dia.

FIG. 9. GIANT CELL FROM THE SAME PREPARATION, WITH LARGE CENTRAL AREA SURROUNDED BY AN INTERMEDIATE ZONE OF FAT GLOBULES AND MITOCHONDRIA, IN WHICH ARE IMBEDDED THE NUCLEI

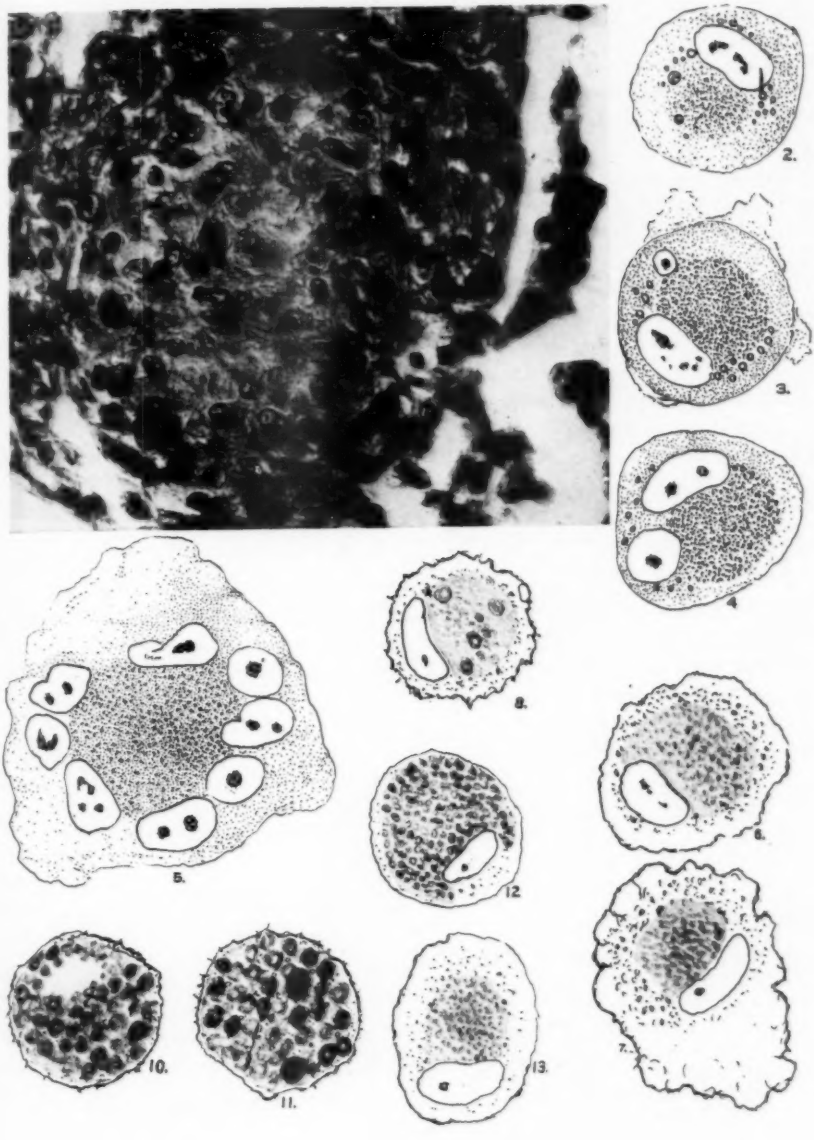
A thin layer of clear cytoplasm surrounds the cell. × 1350 dia.

FIGS. 10, 11, AND 12. CLASMATOCYTES FROM THE SPREADS OF THE NORMAL LUNG

The cells are packed full of bodies which took up the neutral red. × 1350 dia.

FIG. 13. AN EPITHELIOID-LIKE CELL FROM THE SAME PREPARATION

× 1350 dia.


2.
3.
4
8.
5.
12
6.
10.
11.
13.
7.

SPREADS FROM NORMAL LUNGS

The normal lung contains many ordinary clasmatocytes (Figs. 10 to 12) more or less crowded with large, irregularly distributed vacuoles and occasional fat globules. As the clasmatocytes are distributed more or less evenly throughout the lung, only a few were along the cut edges or had dropped out into the surrounding fluid. The large epithelioid cells were entirely absent, but cells resembling young epithelioid cells, probably modified monocytes, were occasionally found (Fig. 13). There were thus marked differences between the cells seen in the normal and those found in the tuberculous lung.

CULTURES OF TUBERCULOUS LUNGS IN AUTOPLASMA

During the first few days after the cultures were made, the great majority of cells that migrated out from the explant were the typical epithelioids, similar to those seen in the spreads of the tuberculous lungs. A few clasmatocytes and blood-cells were seen and an occasional giant cell of the epithelioid type. The older cultures contained, in addition, varying numbers of connective-tissue or endothelial cells which migrated along the coverglass. As the plasma clots liquefied, many of the epithelioid cells settled at the bottom on the surface layer of the drop. Most of these were irregular in form and had the usual short membranous pseudopodia. In the unstained preparations the typical pale granular central area, somewhat variable in size, was easily recognized, as was also the darker, granular, intermediate zone and a clear, thin, peripheral layer with varying numbers of pseudopodia. The nuclei were located in the usual position.

A small grain of neutral red was placed on one side of a culture and one of Sudan III on the other. On the neutral-red side the epithelioid cells in the immediate neighborhood of the dye rapidly accumulated the red color

---

FIGS. 14, 15, AND 16. LARGE MONONUCLEARS FROM ONE-DAY CULTURE OF RABBIT'S BLOOD
× 1350 dia.

FIGS. 17 AND 18. SMALL LYMPHOCYTES FROM SAME CULTURE
× 1350 dia.

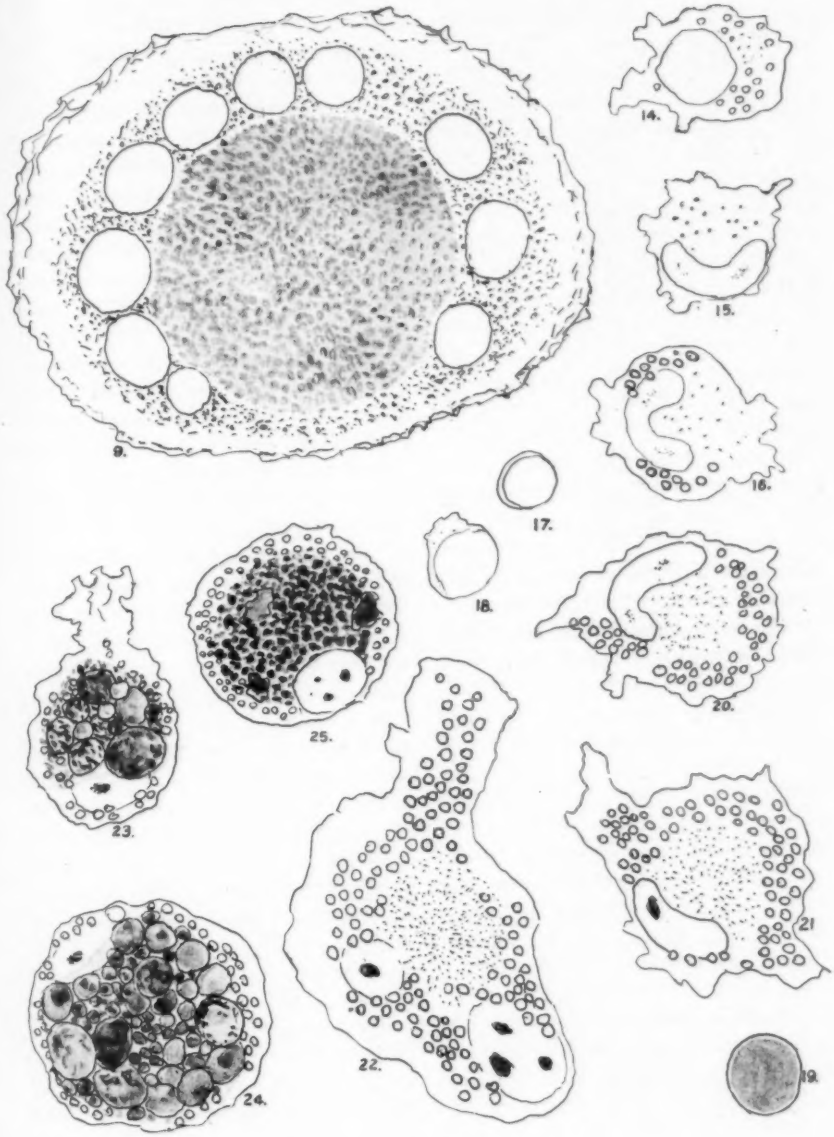
FIG. 19. RED BLOOD-CELL FROM SAME CULTURES
× 1350 dia.

FIG. 20. EPITHELIOID CELL FROM A TWO-DAY BLOOD CULTURE
× 1350 dia.

FIG. 21. EPITHELIOID CELL FROM A THREE-DAY CULTURE OF RABBIT'S BLOOD
× 1350 dia.

FIG. 22. EPITHELIOID CELL FROM A FIVE-DAY CULTURE OF RABBIT'S BLOOD
× 1350 dia.

FIGS. 23, 24, AND 25. MACROPHAGES FROM A FIVE-DAY CULTURE OF RABBIT'S BLOOD
× 1350 dia.


9.
14.
15.
16.
17.
18.
20.
25.
23.
21
22.
24.
19.

in the central area. The more deeply stained cells appeared to have the largest central areas, while the intermediate zone appeared to be reduced in width. A radial arrangement of the granules about a central point was often seen. On the Sudan III side the fat globules, which appeared to make up the bulk of the intermediate zone, slowly assumed a light yellow color. In the more deeply stained cells this zone appeared thicker than in the cells with neutral red. The pale central area did not take the Sudan III.

The nuclei were usually more easily seen in the stained cells than in the unstained ones. They were more or less oval in form and somewhat concave on the side adjacent to the central area. The membranous pseudopodia of the thin peripheral layer often formed a fringe about the cell. There was thus a very exact correspondence in the character of the epithelioid cells in spreads and in cultures.

CULTURES OF NORMAL LUNG IN AUTOPLASMA

The first cells to migrate out into the plasma clot in the cultures of the normal lung were the clasmatocytes, so abundantly present in the explants, and a few white blood-cells. Later, after several days, a few connective-tissue or endothelial cells also migrated out along the coverglass. They remained, however, quite easily distinguishable from the clasmatocytes, even though they became heavily laden with fat globules. The clasmatocytes did not appreciably alter in structure during the period the cultures were under observation. During the later days small epithelioid-like cells were seen, but their origin was not followed.

CULTURES OF NORMAL BLOOD

Following the method of M. R. Lewis (2), cultures were made of normal rabbit's blood by simply preparing hanging drops of whole blood under aseptic conditions. M. R. Lewis found in cultures of avian and mammalian blood that some of the white blood-cells, probably the large mononuclears, underwent hypertrophy, exhibited high phagocytic power, especially for the red blood-cells, and became transformed into clasmatocytes and epithelioid cells. Lewis and Lewis (3) observed even more decided changes in the white blood-cells in cultures of the blood of lower vertebrates—elasmobranchs, teleosts, amphibia, and reptiles. The hypertrophy, phagocytosis, and transformation of the leucocytes (large mononuclears) into clasmatocytes, epithelioid cells, and giant epithelioid cells of the Langhans type, was usually more rapid, more abundant and more remarkable than in the birds and mammals. The resulting clasmatocytes, epithelioid cells, and giant cells, arising in cultures of the blood of these lower forms, resembled in a very striking manner similar cells of the rabbit, and, as already stated, directed our attention to the leucocytic origin of the epithelioid cells in particular.

The transformations in the cultures of the rabbit's blood were neither so abundant nor so marked as in those of the lower vertebrates, but they sufficed to show that both epithelioid cells and macrophages can arise directly from the blood cells. The large

mononuclear forms had often a slight increase in the number of granules and fat globules in the twenty-four hour cultures (Figs. 14, 15, and 16). In the forty-eight hour cultures a few somewhat hypertrophied mononuclears had already taken on the epithelioid appearance (Fig. 20), characterized by an enlarged central area consisting of pale granules more or less radially arranged about a central point (the centrosome) and the accumulation of fat globules into an intermediate zone. Slightly large binucleate cells were occasionally encountered. Some of the older cultures contained, in addition to the epithelioid cells (Figs. 21 and 22), occasional clasmatocyte-like cells (Figs. 23 to 25) with large vacuoles more or less irregularly distributed. The cultures were in rather poor shape after the fifth day, but a few epithelioid cells were seen even on the eighth day. A glance at Figures 14 to 22 indicates how the large mononuclears become transformed into the epithelioid cells.

A few binucleate epithelioid cells were observed, but no multinucleated ones developed in the series of cultures from rabbit's blood. Multinucleated forms, however, were fairly abundant in some of the blood cultures of other animals, more especially those of the lower vertebrates studied by M. R. and W. H. Lewis.

DISCUSSION

The comparison of the epithelioid cells in sectioned material with the living cells in fresh spreads from tuberculous lungs has enabled us to identify the living epithelioid cells. We have noted how different the latter are from the usual familiar histological picture (with the haematoxylin and eosin stain) of the large, somewhat vesicular epithelioid cells that are arranged more or less in layers in the tubercles. Having thus become familiar with the very striking and characteristic living epithelioid cells, we were in a position to appreciate this astonishing similarity to certain of the cells in the blood cultures which we believe to be epithelioid cells.

It is generally accepted by workers in the field of experimental tuberculosis that the developing tubercle is essentially the product of proliferation of epithelioid cells *in situ*. The question now arises as to the source of these early epithelioid cells. At present we are unable to give a definite answer on this point. Further work on the elucidation of this puzzling question is in progress.

The indications are that the epithelioid cells in blood cultures come from the large mononuclears or monocytes. We have not followed the actual transformation, minute by minute or hour by hour, of any single cell, and until this is done some slight doubt might reasonably exist in the mind of the reader as to whether other types of white cells do not take part in this phenomen. It may be that more than one type of leucocyte can undergo this transformation in cultures, but there was no indication that the granulocytes were altered in this manner under the special conditions of our cultures.

Since these cells form in the stagnant hanging drop blood cultures and in cultures of normal human lymph nodes (Lewis and Webster, (4)) there can scarcely be anything chemically specific about the tubercle bacillus that is responsible for this transforma-

tion from blood-cells, as Maximow (1) has suggested.

It has been known for a long time that the centrosome is rather prominent in certain of the blood-cells, and recently Cunningham, Sabin, and Doan (5) have emphasized the existence of a few neutral-red granules about it in the monocyte. There is no particular evidence that the centrosome itself enlarges to form the central area; on the contrary, the granules that compose it are probably cytoplasmic inclusions derived for the most part from ingested stuffs.

The clasmatocytes or macrophages which arise from the monocytes are characterized by many large masses of ingested and partly digested stuffs which more or less completely fill the cell, obscuring the centrosome and often the nucleus. The principal difference between the clasmatocyte and the epithelioid cell seems to depend upon the amount, character, and condition of the ingested stuffs. Hence it is not surprising that both M. R. Lewis and W. H. Lewis (6) have followed the transformation of clasmatocytes into epithelioid cells, and of epithelioid cells into clasmatocytes.

There are thus certain variables in the cytological characters which, by their increase or decrease, account for all the differences between these cells. The most important variable is concerned with the amount, the character, and the condition of the ingested stuffs, and the degree of their digestion. The second is concerned with the accumulation of fat; the third with changes in the nucleus.

CONCLUSION

The epithelioid cells of the older (fourteen-day) tuberculous lesions are similar in appearance to the epithelioid cells formed by the transformation of the monocytes in blood cultures.

REFERENCES

(1) Maximow, A.: *Jour. Inf. Diseases*, 1924, xxxiv, 529.
(2) Lewis, M. R.: *Amer. Jour. Path.*, 1925, i, 91.
(3) Lewis, M. R., and Lewis, W. H.: In preparation.
(4) Lewis, W. H., and L. T. Webster; *Jour. Exper. Med.*, 1921, xxxiii, 349; xxxiv, 397.
(5) Cunningham, Sabin, and Doan: *Proc. Soc. Exper. Biol.*, 1924, xxi, 326.
(6) Unpublished.

Evidence Showing the Existence of Two Distinct Types of Pigment Cells Capable of Giving Rise to Melanotic Tumors[1]

By David T. Smith

From the Department of Embryology, Carnegie Institution of Washington, and the Surgical Pathological Laboratory, Johns Hopkins Hospital

INTRODUCTION

THE malignant pigmented tumors are among the most fatal of all neoplasms. The long series of cases reported by Bloodgood by Coley and Hoguet (1916), Broders and McCarthy (1916), and by others, serve to emphasize the futility of operation when the disease is once well established. Practically the only advance in treatment in this field has been the introduction of the practice, by Bloodgood, of removing suspicious pigmented moles before they become malignant.

It was thought that a study of the cytology of the individual cells, their embryology and their normal distribution throughout the body, as well as the changes produced in the cells when they form benign pigmented moles, might give some new clues as to treatment. Upon attempting to collect the literature on the subject, it was found that one group of authorities claims that all pigmented tumors of the melanin type are epithelial in origin, while another group of equally qualified observers affirms that they are all of mesothelial origin. Certain investigators, among them Ewing and MacCallum, refuse to commit themselves, saying that there is not sufficient evidence at hand on which to base a final decision as to which view is correct. It seems as though a study of the primary pigmented cells of the body might serve to reconcile these conflicting opinions, which arose as a result of studies confined almost exclusively to the fully developed malignant pigmented lesions.

NATURE OF THE PIGMENT

The exact chemical structure of the melanin pigment which gives to these tumors their characteristic color has not been determined. It is not derived from hemoglobin and does not contain iron (Abel and Davis, 1896). The granules which are seen with the microscope are supposed to consist of protein molecules which have pigment groups attached (Abel and Davis, 1896; Smith, 1920). The pig-

[1] Received for publication October 16, 1924.

ment group was considered for a long time to be either tyrosine or some substance closely related to it, because such compounds react with the enzyme tyrosinase to produce a black pigment (Bertrand, 1908; Von Furth, 1901). Block isolated dioxyphenylalanin from the embryos of the broad bean and found that this chemical substance reacts with normal non-pigmented skin to produce granules of pigment which seem to be identical with natural melanin. So striking is this reaction that he employs it as a histological stain to differentiate cells capable of producing melanin pigment (such as the cells in the basal layer of the skin) from those which never produce pigment. This is the so-called "Dopa" reaction (Block, 1921, 1923; Spencer, 1923).

Some comprehensive chemical work on the nature of melanin pigment has been undertaken by Brahn and Schmidtmann (1920). Brahn insists that all natural melanin contains sulphur. Schmidtmann found that melanin induces vaso-constriction as does adrenalin, but has no effect on the heart and does not produce hyperglycemia. It has been the belief for a long time that there must be some relation between the adrenals and pigment metabolism, because of the occurrence of melanin pigment in the skin in considerable amounts in Addison's disease. MacLachlan (1915) reported an interesting case in which both adrenals were almost completely destroyed by a melanotic sarcoma, and yet there was none of the typical Addisonian pigmentation of the skin. He assumed that the growing pigment cells utilized all of the available melanogen, leaving none to be deposited in the cells of the skin. On the other hand, melanuria may occur in the absence of a melanotic tumor and in the absence of adrenal destruction (Peters, 1923; Haden and Orr, 1924). Our knowledge of pigment chemistry will not be complete until we can correlate the abnormal production of melanin with that which goes on as a physiological process in the skin of the negro.

PIGMENT CELLS IN TISSUE-CULTURES

Luna (1917) cultivated the pigmented epithelium of the retina of the embryo chick's eye in plasma cultures. He noted that the cells grew in sheets like epithelium, an observation which I have been able to confirm (Smith, 1920), for when bits of this pigmented membrane were planted in Locke-Lewis solution, the pigmented cells grew out on the under surface of the cover-glass just as epithelium does. The melanin granules in the cells were quite large, about 1 micron in length, and definitely rod-shaped. By cultivating cells from the eyes of very young embryos (four days' incubation) it was possible to get some idea as to the origin of the granules. Apparently they arise in the cytoplasm as small colorless or grayish granules, which grow larger and darker until the mature form is reached. All the evidence obtained by observation on the living unstained cells and by the use of supravital staining indicates that the granules do not arise from the mitochondria as claimed by Luna (1917), or from the nucleus, as affirmed by von Szily (1911) and others.

All the pigmented cells of the retina proper have rod-shaped granules, but in the retinal part of the iris, which is

merely a continuation of the retina beyond the ora serrata, the cells contain large round granules. When cultures of these cells are grown they also give the characteristic appearance of epithelial tissue. Indeed, cultures of these cells have been grown by successive generations for over three months at the Rockefeller Institute, and they still retain their epithelial character (Fisher, 1922).

In the choroid, iris, and ciliary body one finds scattered isolated pigmented cells of a quite different appearance from those described above. They have long processes which are often branched. The cells are packed with small round granules which fill the cytoplasm completely, even out to the very tips of the processes. While the present study was in progress, a research was published by Luna (1920) in which he showed that in plasma cultures these cells from the choroid grew as isolated cells, like the fibroblasts, and not in sheets like epithelium. Cultures made in this laboratory in Locke-Lewis solution showed the same characteristics; it was quite evident that we were dealing with two distinct types of pigment cells—one behaving as epithelium, the other as connective tissue. Even though the cells were so different in appearance and showed such marked variation in the size and shape of the granules, one was impressed with the fact that the granules, in any particular cell under observation, were always about the same size, shape, and color, and were evenly distributed throughout the cytoplasm. This picture was constant for those cells that had produced their own pigment.

INGESTION OF PIGMENT BY TISSUE-CULTURE CELLS

When granules of pigment were taken in by cells not normally pigmented, the picture was distinctly different from that described above. In this case the pigment was a foreign body in the cytoplasm and the cell reacted in such a manner as to clump it into masses of irregular size and shape. Cultures of various types of embryonic chick cells were grown and pigment granules then added to the culture fluid. The whole process of ingestion and clumping of the granules into masses in the cytoplasm, with the formation of a vacuole about each mass, could be followed with the microscope (Smith, 1921). It was found that white blood-cells, clasmatocytes, connective-tissue cells, mesothelial cells, endothelial cells, alveolar cells of the lung, tubule cells of the kidney, liver cells, Kupffer cells, and even the epithelial cells of the amnion were able to ingest pigment. The granules were not taken in by the red blood-cells, striated-muscle cells, contracting smooth-muscle cells, or peripheral-nerve cells. The reaction was always the same, regardless of the source of the pigment. Melanin granules from the eye (chick, pig, dog, or human), carbon particles, carmine granules, India ink, and blood pigment, all became clumped in the cytoplasm into masses of irregular size and shape. When once the pigment had been taken into the cytoplasm it was not given up until the death of the cell.

It is evident from these observations that here is a practical method of determining from the appearance of the granules, *when examined with the*

oil immersion lens, whether they have been produced by the cell or have been taken in as foreign bodies. In studying the pigment cells we need no longer be confused by the question whether a particular cell under observation is a *pigment-producing* or a *pigment-carrying* cell.

INGESTION OF PIGMENT BY TUMOR CELLS

Observations on sections from 200 pigmented tumors of the melanotic type and 160 tumors of various kinds containing blood pigment showed that the principles worked out in tissue-cultures are equally applicable to the ingestion of pigment by the cells of the living body. It is generally possible to tell, even in the most complicated cases, which cells have produced the pigment and which have taken it in as a foreign body. The only difficulty likely to be encountered is the fact that the true melanin pigment in the dead degenerate areas of the tumor may appear in clumps and masses like ingested pigment. But in every tumor examined in which these degenerate areas were present there could be found, in other parts of the section, areas in which the pigment cells were in good condition and showed the characteristic picture of true endogenous pigment. The method is so simple that it can be used in the diagnosis of pigmented lesions at the time of the operation by a study of frozen sections (Smith, 1924).

DISTRIBUTION OF PIGMENT CELLS THROUGHOUT THE BODY

The study of pigmented cells in tissue-cultures indicates that there are two distinct types of cells which produce the pigment present in their cytoplasm. These types are found in definite regions of the body. In the retina and the retinal part of the iris one finds pigmented cells of the epithelial type. In the retina proper the granules are large, rod-shaped bodies, about 1 micron in length and $\frac{1}{4}$ micron in width, while in the retinal part of the iris they are round or oval and about $\frac{1}{4}$ micron in diameter. The choroid, iris, and ciliary body show the connective-tissue type of pigmented cell. These are more or less isolated cells with long processes which often branch. The granules are small and round and about one-fourth the size of the round granules of the retinal part of the iris. The basal layer of the skin in the darker races contains small, short, rod-shaped granules of melanin pigment, one-fourth to one-third as large as those in the retina. The matrix of the hair follicles is composed of epithelial cells which are generally filled with small rod-shaped granules, like those seen in the skin. The large nerve-cells of the *substantia nigra* and *locus caeruleus* always contain pigment of the melanin type. The granules are quite black and have a tendency to be rod-shaped. They resemble those in the skin and hair follicles, although they are a little larger. The mongolian spot, found over the sacrum in members of the mongolian and negro races, is due to cells of connective-tissue type in the dermis.

In addition to these locations where pigment is found constantly, there are other places where it is observed occa-

sionally. The epithelial type of pigmented cell may be found in the mouth, on the, vulva or in the rectum. In the dermis, generally entirely separated from the epithelium and the hair follicles, one may find small isolated masses of long, spindle-shaped cells of the connective-tissue type. occur in the human adrenal also (Lucksch, 1912; Tuczek, 1914). In dermoids of the ovary (Fig. 2) pigmented cells of the connective-tissue type may be found and also epithelial cells that are probably capable of producing pigment. Dermoids in other parts of the body may have the

FIG. 1. (GEN. PATH. NO. 7332.) PIGMENTED CELL OF THE CONNECTIVE TISSUE TYPE FROM THE MENINGES OF A NEGRO CHILD. (×2600)

FIG. 2. (GYN. NO. 27487.) CONNECTIVE-TISSUE TYPE OF PIGMENTED CELL FROM A DERMOID OF THE OVARY. (× 3000)

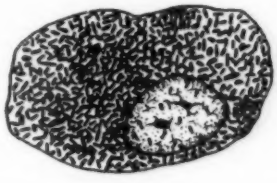


FIG. 3. (SURG. PATH. NO. 5028.) PIGMENT CELL OF THE EPITHELIAL TYPE REGULARLY FOUND IN THE PIGMENTED LAYER OF THE RETINA (× 2600)

This cell was drawn from a section of a partially developed eye found in a teratoma located in the sacrum of a child.

Pigment cells morphologically identical with those of the choroid are occasionally observed scattered along the meninges of the brain (Fig. 1). This is much more common in the negro than in the white race. In the adrenal capsule of the guinea-pig the connective-tissue type of pigmented cells may be found, the same type may same properties. The teratomata (Fig. 3) likewise may supply pigmented cells to a region which otherwise would contain none.

With the exception of the nerve cells of the brain, it is possible to find reports in the literature of pigment tumors arising from all of the regions known to have pigment, either con-

stantly or occasionally.[2] A corollary to this is that pigment tumors probably arise only from actual or potentail pigment cells.

PIGMENTED CELLS IN THE HUMAN EMBRYO

It was decided to make a survey of the collection of human embryos belonging to the Department of Embryology of the Carnegie Institution of Washington, to see if there was any evidence to indicate that the connective-tissue type of pigment cell might arise by metaplasia from the epithelial type or vice versa, or whether both were derived from a common parent cell.

The rod-shaped granules of the pigmented epithelium of the retina make their appearance at about the end of the first month of intrauterine life; the round granules of the retinal part of the iris appear at the same time or a few days later, while the small round granules found in the cells of the choroid, iris, and ciliary body appear very much later, from the fifth to the seventh month, generally about the seventh. The process of pigmentation in these cells continues after birth and manifests itself in some cases by changing the blue iris of the newborn child into a brown or black one during the first few months of life (Bach and Seefelder, 1914). The cells responsible for the mongolian spot are supposed to become pigmented about the fourth month of intrauterine life (Adachi, 1902). Pigment appears in the cells forming the matrix of the hair of the fetus about the sixth month, before there is any pigment in the skin above. The pigment in the epithelial cells of the skin appears very late, generally just before birth.

It is known that the pigment cells of the retinal part of the iris "bud" down into the mesothelial tissue beneath, lose their pigment granules, and form the muscles of the iris—musculus sphincter and musculus dilator pupillae (von Szily, 1902; Lewis, 1903). Sections of this region were studied to ascertain if other pigment cells from the epithelial layer might not be changed into pigment cells of the connective-tissue type. The "budding" of the epithelial cells, with the loss of the large round granules and the formation of muscle, could be followed, but there was no evidence that these cells give rise to the pigmented cells of the iris or of the choroid (Fig. 4). No transitional stages could be found, either in the type of cell or the character of the granules. It was quite clear that the connective-tissue type of pigmented cell arises *in situ* entirely distinct and separate from the epithelial type. At first the granules are very small and light tan in color, but as the cell becomes mature the granules become larger and darker.

BENIGN PIGMENTED MOLES

The embryological and histological studies, as well as the experiments with tissue-cultures, have demonstrated that there are two types of cells in the human body capable of producing melanin pigment. The next step was to determine which type of pigment cell gives rise to the benign pigmented moles of the skin. There

[2] See chapter on *Melanoma*, in "Neoplastic Diseases—A Text-book on Tumors," by James Ewing, 1919, W. B. Saunders Co., Philadelphia and London.

were available for this study, in the Laboratory of Surgical Pathology, sections from 250 benign moles. An analysis of the sections showed that 240, or 96 per cent of them, were composed of cells of the epithelial type. These were the ordinary moles with which every one is familiar. This finding confirms the conclusions of Unna (1893), Gilchrist (1899), and

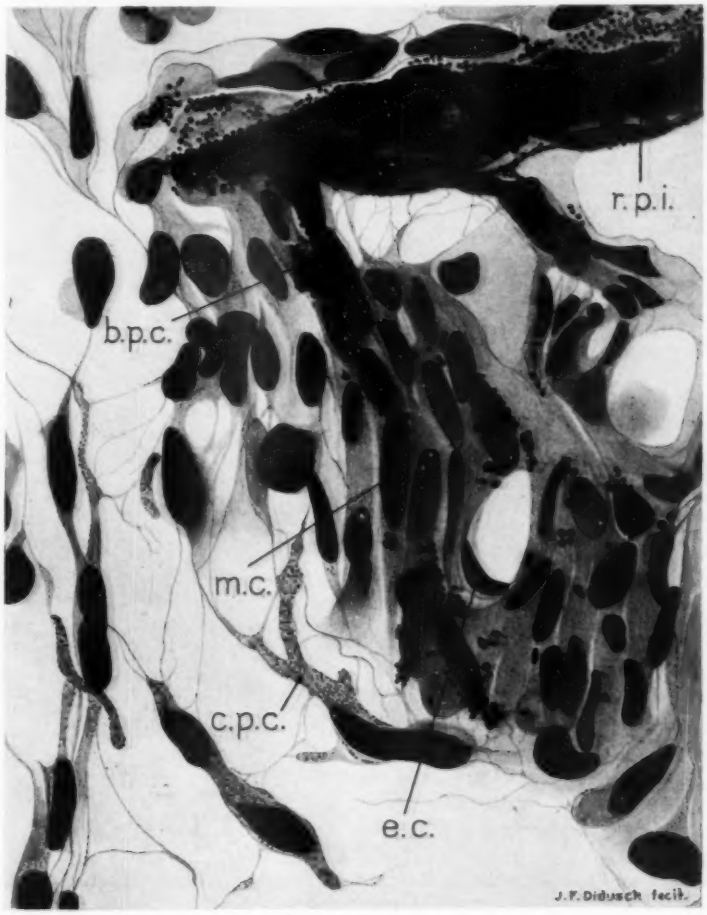


FIG. 4. SECTION THROUGH THE REGION OF THE CILIARY PROCESS OF THE EYE OF A HUMAN FETUS (C.C. No. 3800, 250 MM. C. R. LENGTH, AGE ABOUT TWENTY-EIGHT WEEKS), SHOWING BUDDING OF EPITHELIAL CELLS OF IRIS (b.p.c.) AND THEIR DIFFERENTIATION INTO MUSCLE CELLS (m.c.)

It will be noticed that they can be clearly distinguished from the pigment cells of the choroid (c.p.c.) and the pigment cells of the iris (r.p.i.). e.c., endothelial cell.

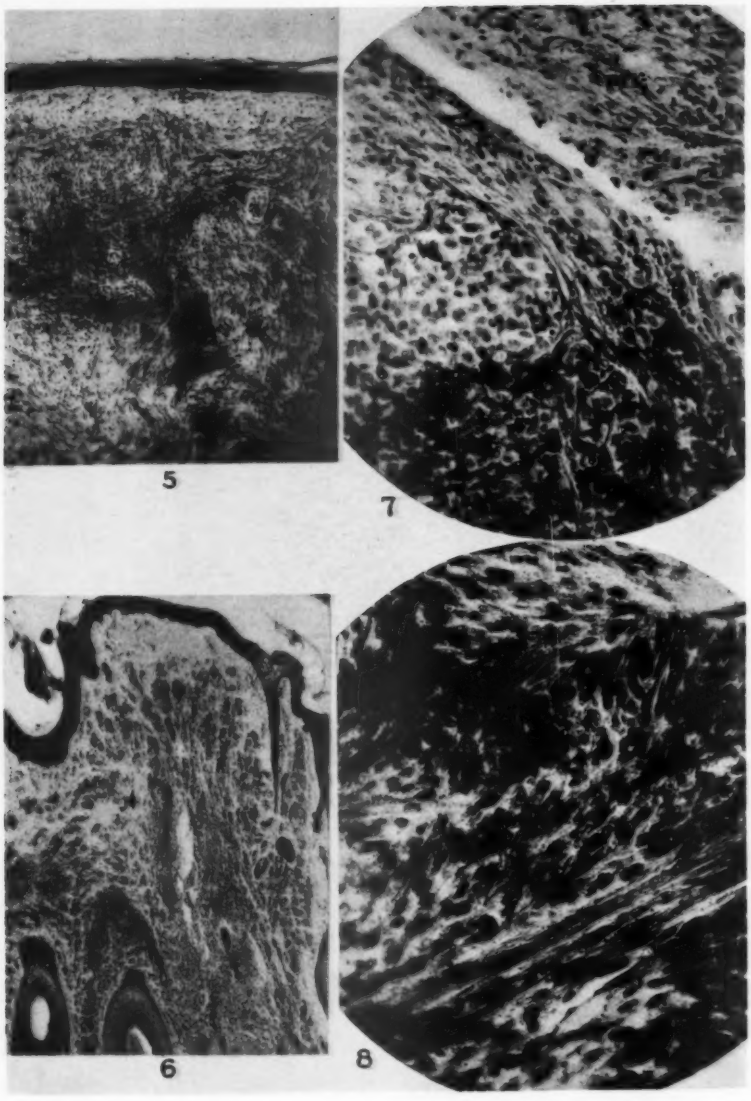


FIG. 5. (SURG. PATH. NO. 10370.) PHOTOMICROGRAPH OF A BENIGN PIGMENTED MOLE OF THE CONNECTIVE-TISSUE TYPE

The epidermis is normal, the lesion being in the dermis

FIG. 6. (SURG. PATH. NO. 2167.) PHOTOMICROGRAPH OF A BENIGN PIGMENTED MOLE OF THE EPITHELIAL TYPE

The lesion is primarily of the epithelial layer of the skin, although hair follicles and sweat glands also show changes.

FIG. 7. (SURG. PATH. NO. 16,601.) PHOTOMICROGRAPH OF A MELANO-CARCINOMA OF THE SKIN

Figure 13 is a drawing of one of the cells in this section

FIG. 8. (SUR. PATH. NO. 21,006.) PHOTOMICROGRAPH OF A MELANO-SARCOMA OF THE SKIN

Figures 14 and 15 are drawings of cells in this tumor

Dalla Favera (1908) that the common moles of the skin are epithelial in origin (Figs. 6 and 10).

Ten specimens (or 4 per cent of the total number) showed an entirely different picture. The lesion was in the dermis, beneath normal non-pigmented epithelium, and was composed of long, spindle-shaped cells, some of which showed branching processes. These cells were packed with small round granules of pigment, all of which were about the same size, shape, and color (Figs. 5 and 9). The morphology of the cells suggests that they are identical with the pigmented cells of the choroid and are probably the "chromatophores" which Ribbert

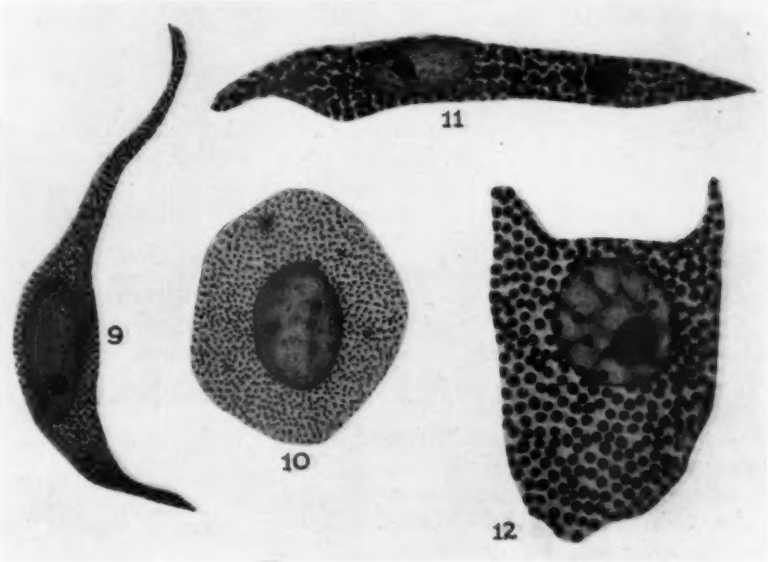


FIG. 9. CAMERA-LUCIDA DRAWING OF ONE OF THE CELLS FROM FIG. 5. (× 2,600)

The granules are small, round, and evenly distributed throughout the cytoplasm

FIG. 10. CAMERA-LUCIDA DRAWING SHOWING A PIGMENTED CELL OF THE EPITHELIAL TYPE FROM FIGURE 6. (× 2600)

The granules are very small and are evenly distributed throughout the cytoplasm. They are not perfectly round, as in Figure 9, but show a tendency to be rod-shaped.

FIG. 11. CAMERA-LUCIDA DRAWING OF A CONNECTIVE-TISSUE CELL FROM FIGURE 6. (× 2600).

This cell has taken in pigment liberated from cells like Figure 10. The pigment is massed into granules which vary greatly in size and shape.

FIG. 12. (VERHOEFF NO. 1648.) CAMERA-LUCIDA DRAWING OF A MALIGNANT CELL WHICH AROSE FROM THE RETINAL PORTION OF THE IRIS. (× 2600)

These large round granules are found nowhere in the body except in cells from this portion of the eye.

(1897) and his coworkers erroneously considered to be the cause of all pigmented moles. This type can be recognized clinically as a smooth, bluish black discoloration of the skin. The peculiar bluish color is due to the fact that the pigmented cells are located in the dermis beneath several layers of normal epithelial cells. Palpation generally reveals a definite circumscribed subepidermal nodule.

Ingested pigment could be found in both types of benign pigmented moles. This pigment was recognized by its characteristic occurrence in masses of different sizes and shapes (Fig. 11). Some of it was probably derived from hemoglobin, but for the most part it was ingested melanin which did not give the iron reaction. Spindle-shaped cells containing these masses of pigment were frequently found in the epithelial type of mole, also round cells in the connective-tissue type. It would have been impossible to differentiate the two types of benign moles had I not learned to distinguish the cells which take in pigment as a foreign body from those which produce it in their own cytoplasm (Smith, 1924).

MALIGNANT PIGMENTED TUMORS OF THE SKIN

Among 100 malignant moles of the skin 67 were found to be of the epithelial type. These tumors behaved in general much like an ordinary carcinoma (Figs. 13 and 7). They metastasized to the regional glands, liver, brain, skin, and occasionally even to the bones. The remaining 33 malignant tumors were definitely sarcomatous in character. They were generally of the spindle-cell type or mixed, spindle- and round-cell, although other forms of sarcomatous growth were represented (Figs. 14 and 8). These tumors tended to occur in younger patients and metastasized to the liver, lungs, brain, and glands, but not to the skin or bones. The origin of most of the melanosarcomas could be traced to a benign pigmented mole of the connective-tissue type.

Ingested pigment could be found in the malignant lesions even more frequently than in the benign ones (Fig. 15). In a number of cases large amounts of ingested malanin pigment could be seen in the connective-tissue stroma of a melanocarcinoma, while the tumor cells proper contained very little pigment.

MALIGNANT PIGMENTED TUMORS OF THE EYE AND OTHER PARTS OF THE BODY

A large proportion of the pigmented tumors of the eye are of the sarcoma type (80 per cent of 30 cases)[3] and appear to originate from the pigment cells of the choroid, iris, and ciliary body. (For literature, see Mannheimer, 1922.) Some, however, are epithelial in character and begin in the retinal portion of the iris. One such specimen showed the large, round pigment granules which are to be encountered nowhere in the body except in cells from this portion of the eye (Fig. 12). In one case (Surg. Path. No. 25812) sections of one of the skin metastases showed cells of the epithelial type filled with pigment granules,

[3] Most of these specimens were obtained for study through the kindness of Dr. C. A. Clapp of Baltimore, Dr. Frank N. Irwin of New York, Dr. F. H. Verhoeff of Boston, and Dr. Alan Woods of Baltimore.

some of which were definitely rod-shaped. The tumor probably originated from cells of the pigmented layer of the retina, although this could not be proved, because of the failure to obtain the primary ocular lesion.

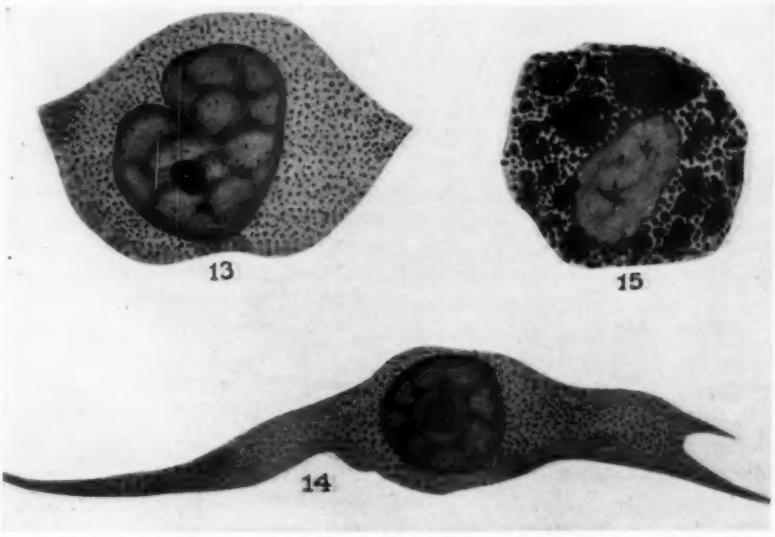


FIG. 13. (SURG. PATH. NO. 16,601.) CAMERA-LUCIDA DRAWING OF A MALIGNANT CELL FROM A MELANO-CARCINOMA OF THE SKIN. (× 2600)

The granules of pigment are very small, dust-like particles. Figure 7 is a photomicrograph of the section from which this cell was drawn.

FIG. 14. (SURG. PATH. NO. 21,006.) CAMERA-LUCIDA DRAWING OF A MALIGNANT CELL FROM A MELANO-SARCOMA OF THE SKIN. (× 2600)

With the development of malignancy the granules have become very small and dust-like. The section in which this cell occurred is shown in Figure 8.

FIG. 15. (SURG. PATH. NO. 21,006.) CAMERA-LUCIDA DRAWING OF A PHAGOCYTIC CELL FOUND IN THE SAME TUMOR. (× 2600)

These granules have been taken in as foreign particles and seem to be in masses which vary greatly in size and somewhat in shape. Note the marked difference in appearance between these granules and those seen in Figures 12, 13, and 14.

Melanomas from other parts of the body may be either epithelial or of the connective-tissue type, depending upon the pigment cell from which they originate. Tumors of the epithelial type were found in the caecum, the rectum, the ovary, and the testicle; of the connective-tissue type in the ovary and in the adrenal.[4] Some cases will be encountered where it is not possible to make an absolute diagnosis of melanosarcoma or melanocarcinoma, but the difficulty is neither greater than nor different from that encountered in trying to differentiate

[4] The privilege of studying sections of the melanoma of the caecum was accorded me by Dr. James Ewing, the melanoma of the testicle by Dr. Jean Firket, melanoma of the adrenal by Dr. Alfred Plaut.

certain non-pigmented sarcomas from carcinomas.

DISCUSSION OF TREATMENT

The connective-tissue type of pigment cell was found to give rise to only 4 per cent of the benign moles of the skin, yet 33 per cent of the malignant lesions were of the sarcoma type. Since they are so prone to become malignant, it would seem to be advisable to excise, with a wide margin of normal tissue, every one of these connective-tissue moles as soon as they are discovered, without waiting for evidence of degeneration.

A study of the histories in the fatal cases indicates that all moles which give evidence of growth, increase in pigmentation, infection, ulceration, or bleeding should be removed at once.

Many of the benign moles in this collection showed two or even three of these danger signals, yet the remarkable consistency with which these symptoms are followed by malignancy leaves no doubt that the proper treatment is early and complete excision of the precancerous lesion.

I wish to express my thanks to Dr. and Mrs. Lewis and to Dr. Bloodgood, in whose laboratories these observations were carried out; also to Dr. Streeter for his aid and suggestions in the study of the histogenesis of the human eye.

CONCLUSIONS

1. There are two types of cells in the body capable of producing melanin pigment. In tissue cultures the one grows like epithelium, the other like connective tissue.

2. Cells that have produced pigment may be differentiated from those that have taken in pigment as a foreign body by an examination of the granules with the *oil immersion lens*. The granules are always about the same size, shape, and color when produced in the cell. When taken in as foreign particles they are found in masses which vary greatly in size and somewhat in shape.

3. Normally, the epithelial type of pigment cell can be found in the basal layer of the epithelial coat of the skin, hair follicles, retina, and certain regions of the brain. The connective-tissue type of pigmented cell is found in the choroid of the eye, iris, ciliary body, and in the "mongolian spot," when that is present.

4. Occasionally the epithelial type is found in the mouth, rectum, dermoids, and in teratomata; the connective-tissue type in the dermis, along the meninges of the brain, about the adrenal gland, and in dermoids.

5. Observations on pigment cells in the human embryo show that the two types arise independently and in different stages of embryonic development.

6. Among 250 benign moles of the skin, 96 per cent were of the epithelial and 4 per cent of the connective-tissue type.

7. Among 100 malignant pigmented moles of the skin, 67 per cent were of the epithelial and 33 per cent of the connective-tissue type.

8. Among 30 tumors of the eye, 20 per cent were of the epithelial and 80 per cent of the connective-tissue type.

9. Tumors arising in other parts of the body were either epithelial or connective tissue, depending upon the type of pigment cell from which they originated.

REFERENCES

ABEL, J. J., AND W. S. DAVIS 1896 On the pigment of the negro's skin and hair. Jour. Exper. Med., i, 361.

ADACHI, B. 1902 Hautpigment beim Menschen und beim Affen. Anat. Anz., xxi, 16.

BACH, L., AND R. SEEFELDER 1914 Atlas zur Entwicklungsgeschichte des menschlichen Auges.

BERTRAND, G. 1908 Recherches sur la mélanogénèse: Action de tyrosinase sur la tyrosin. Ann de l'Inst. Pasteur, xxii, 381.

BLOCK, B. 1921 Über die Entwicklung des Haut- und Haarpigmentes beim menschlichen Embryo und über das Erlöschen der Pigmentbildung im ergrauenden Haar. Arch. f. Dermatol. u. Syph., cxxxv, 77.

BLOCK, B. 1923 Der jetzige Stand der Pigmentlehre. Zentralbl. f. Haut. u. Geschlechtskr., viii, 1.

BRAHN, B., AND M. SCHMIDTMANN 1920 Zur Kenntnis des Melanins und des braunen Abnutzungspigments. Virchows Arch., ccxxvii, 137.

BRODERS, A. C., AND W. C. MACCARTY 1916 Melano-epithelioma. Report of 70 cases. Surg., Gyn., and Obst., xxiii, 28.

COLEY, W. B., AND J. P. HOGUET 1916 Melanotic cancer, with a report of 90 cases. Trans. Amer. Surg. Assn., Philadelphia, xxxiv, 319.

DALLA FAVERA, G. B. 1908 Ein Beitrag zur Kenntnis der Pigmentnaevi. Ziegler's Beiträge, xliii, 43.

EWING, JAMES 1919 Neoplastic Diseases. A Text-Book on Tumors. W. B. Saunders Co., Philadelphia and London.

FISHER, A. 1922 Three months old strain of epithelium. Jour. Exper. Med., xxxv, 367.

VON FURTH, O., AND H. SCHNEIDER 1901 Ueber tierische Tyrosinasen und ihre Beziehungen zur Pigmentbildung. Beitr. z. Chem. Physiol. u. Path., i, 229.

GILCHRIST, C. T. 1899 Are malignant growths arising from pigmented moles of a carcinomatous or sarcomatous nature? Jour. Cut. and Genito-Urinary Dis., xvii, 117.

HADEN, R. L., AND T. G. ORR 1924 Melanuria in the absence of melanotic tumor. Johns Hopkins Hospital Bull., xxxv, 58.

LEWIS, W. H. 1903 Wandering pigmented cells arising from the epithelium of the optic cup, with observations on the origin of the M. sphincter pupillae in the chick. Amer. Jour. Anat., ii, 405–416.

LUNA, E. 1917 Note citologiche sull epitelio pigmentato della retina coltivato "in vitro." Arch. ital. di Anat. e di Embriol., xv, 543.

LUNA, E. 1920 Studio sulle cellule pigmentate della coroide coltivate in vitro. Arch. ital. di Anat. e di Embriol., xviii, 146.

LUCKSCH, F. 1912 Über pigmentierte Adenome der Nebennieren. Beiträge z. path. Anat. u. z. allg. Path., liii, 324.

MACCALLUM, W. G. 1917 A Text-book of Pathology. W. B. Saunders Co., Philadelphia and London.

MACLACHLAN, W. W. G. 1915 Primary melanosarcoma of the adrenals. Jour. Med. Research, xxxiii, 93.

MANNHEIMER, M. 1922 Die Literatur des letzten zehn Jahre über die bösartigen Tumoren des Auges. Ztschr. f. Krebsforschung, xviii, 1.

PETERS, J. P. 1923 Melanuria without melanosarcoma. Report of a case. Arch. Int. Med., xxxii, 709.

RIBBERT, H. 1897 Über das Melanosarkom, Ziegler's Beiträge, xxi, 471.

SMITH, D. T. 1920 The pigmented epithelium of the embryo chick's eye studied in vitro and in vivo. Johns Hopkins Hosp. Bull., xxxi, 239.

SMITH, D. T. 1921 Ingestion of melanin pigment granules by tissue cultures. Johns Hopkins Hosp. Bull., xxxii, 240.

SMITH, D. T. 1924 Method for making a differential diagnosis between xanthomatous and melanin tumors from frozen sections. Arch. Surgery, viii, 908.

SPENCER, W. G. 1923 Melanosis. British Med. Jour., ii, 907.

VON SZILY, A. 1902 Beitrag zur Kenntnis der Anatomie und Entwicklungsgeschichte der hinteren Irisschichten, mit besonderen Berücksichtigung des Musculus sphincter pupillae des Menschen. Arch. f. Ophthalm., liii, 459.

VON SZILY, A. 1911 Ueber die Entstehung des melanotischen Pigmentes im Auge des Wirbeltierembryonem und im Choroideal-Sarkomen. Arch. f. mikr. Anat., lxxvii, 687.

TUCZEK, K. 1914 Über die Beziehungen der Nebennierenpigmentation zur Hautfarbe. Ziegler's Beiträge, ccclviii, 250.

UNNA, P. G. 1893 Naevi und Naevocarcinome. Berl. kl. Wochenschr., xxx, 14.

Report of the Recovery of T. Pallidum from the Spinal Fluid of a Patient with Syphilitic Meningitis of the Neuro-Recurrence Type

By Jarold E. Kemp and Alan M. Chesney

From the Medical Clinic of the Johns Hopkins Hospital

THE instances in which Treponema pallidum has been isolated from the spinal fluid of patients with an acute syphilitic meningitis of the neuro-recurrence type are sufficiently rare to warrant the report of an additional case. The term neuro-recurrence is understood to mean the occurrence of symptoms and signs of central nervous system involvement, usually of the type associated with acute syphilitic meningitis, and generally accompanied by a paralysis of one or more of the cranial nerves, appearing only in patients with syphilis who have received insufficient treatment in the early stages of the disease and who, prior to the onset of symptoms, have shown no signs of central nervous system involvement. Occasionally, however, the lesion is a focal one without any changes in the spinal fluid suggesting the existence of a meningitis.

In 1913 Nichols and Hough (1) inoculated a rabbit with the spinal fluid from such a patient and later, when the animal had developed an orchitis, were able to demonstrate T. pallidum in the affected testicle. Since then, several other investigators have obtained similar results in such cases by rabbit inoculation or by dark field examination of the fluid. The important facts in each of these cases are given in Table I.

The history of the case we wish to report is as follows:

White, male, aged twenty-two, primary sore six months before admission, treated with two doses of arsphenamine. Three months later acute syphilitic meningitis of the neuro-recurrence type. Paralysis of the left second, seventh, and eighth cranial nerves. Spinal fluid positive. Successful rabbit inoculation.

The patient, a white male, twenty-two years of age, was admitted to the Hospital, August 14, 1923, complaining of deafness in the left ear and paralysis of the left side of his face. His family history was unimportant. In his past history he admitted a genital sore two years previous to admission. It was painful and accompanied by a left suppurative inguinal adenitis. Under local treatment the sore healed in about two weeks. There was no history of any secondary manifestations following this lesion. Six months prior to admission he contracted a second genital sore. It was single, small, hard, painless and accompanied by inguinal adenitis. A blood Wassermann one week later was negative. The following week a second Wassermann was also negative. No dark field examina-

TABLE I

AUTHOR	STAGE OF DISEASE ON FIRST ADMISSION	DURATION OF DISEASE ON FIRST ADMISSION	BLOOD WASSERMANN REACTION ON FIRST ADMISSION	INITIAL TREATMENT		DURATION OF LAPSE	DURATION OF SYMPTOMS OF RECURRENCE
				Arsphenamine	Mercury		
(1) Nichols and Hough, 1913	Secondary, early	About 60 days	Positive	Intravenous injection (0.6 gram) and a second (0.6 gram) after 3 weeks	None	About 140 days	38 days
(2) Reasoner, 1916	Primary	57 days	Positive	2 intravenous injections of neoarsphenamine. Total 1.8 gram	24 intramuscular injections of mercury salicylate	About 42 days	About 21 days
(3) Wile, 1917			Positive	Not given	Not given	Not given	Not given
(4) Joers, 1920	Secondary, early		Positive	None	8 intramuscular injections of mercury salicylate	28 days	1 day
Kemp and Chesney, 1924	Primary	14 days	Negative	Intravenous injection, and a second after 3 weeks	None	120 days	28 days

(5) Uhlenhuth and Mulzer (1913) reported the isolation of treponemes by rabbit inoculation from the spinal fluid of a patient with an acute neuro-recurrence meningitis. Except for a slight lymphocytosis the fluid was negative.

(6) Levy has demonstrated the organisms by dark field examination in the spinal fluid of a patient with an acute syphilitic meningitis. The details of the case are not known.

(7) Ivers also demonstrated the organisms by dark field examination of the spinal fluid of a patient with an acute syphilitic meningitis. Reference not available.

DURATION OF DISEASE TO SECOND ADMISSION	SYMPTOMS AND PHYSICAL FINDINGS ON SECOND ADMISSION	BLOOD WASSERMANN REACTION	SPINAL FLUID				METHOD OF DEMONSTRATION OF T. PALLIDUM
			Cells	Globulin	Wassermann reaction	Gold curve	
About 240 days	Paralysis right leg. Memory and speech defects	Positive	510	Inc.	Negative		Rabbit inoculation. Incubation period 50 days
About 212 days	Headaches, convulsions, loss of consciousness	Positive	184	++	Positive	Syphilitic	Rabbit inoculation. Incubation period (?)
Not given	Headaches and edema of optic discs	Positive	259	++	Positive		Rabbit inoculation. Incubation period 12 days (?)
70 days	Headaches	Positive	340	+++	Positive	Paretic	Dark field examination of centrifugalized fluid.
176 days	Headaches, L. VII, VIII nerve paralysis, edema of l. optic disc	Negative	1730	++++	Positive	Low paretic	Rabbit inoculation. Incubation period 68 days (−)

tion was made. Following this he was given two doses of an unknown amount of arsphenamine separated by an interval of three weeks, and discharged as cured. This treatment was carried out in another city. There was no history of any secondary manifestations at any time. The present illness began about four weeks before admission, and about three months after his last treatment, with a sudden left facial paralysis which improved during the following two weeks. About this time he noticed gradually increasing deafness in his left ear. This was accompanied by marked tinnitus and vertigo which was so severe that he had difficulty in walking. There was no nausea nor vomiting. No symptoms referable to disturbance of the other cranial nerves were noticed by the patient. One week before admission he was attacked with very severe headaches which were constant, with nocturnal exacerbations.

The following important findings were noted on physical examination: The patient was obviously in extreme discomfort as a result of headache. His temperature was 98.2°F., pulse 66, blood pressure 118/74. His pupils were equal but a little irregular. The right one reacted promptly to light and on accommodation, but the left reacted sluggishly and incompletely to light. The extra-ocular movements were well performed. The right fundus was normal. The outline of the left disc, however, was indistinct, with slight edema of the surrounding retina and partial obliteration of the physiological cupping. There was almost complete deafness in the left ear. The vestibular apparatus was not tested. Hearing in the right ear was apparently normal. There was an incomplete, central left facial paralysis. The other cranial nerves appeared normal. The skin and mucous membranes were clear throughout, but there was a well-marked general glandular enlargement. All of the deep and superficial reflexes were more active on the left than on the right. Kernig's sign was positive. The blood Wassermann reaction was negative in both the water-bath and ice-box. The spinal fluid examination showed 1730 cells, 80 per cent of which were lymphocytes, and a four-plus globulin. The Wassermann reaction was completely positive with 0.6 cc. of fluid in the water-bath, and the colloidal mastic curve read 3321000000. A complete blood examination showed the hemoglobin, red blood cells and leucocytes to be within the normal.

His treatment and course in the Hospital were as follows:

August 14 and 23. Neoarsphenamine, 0.9 gram.

September 3. Neoarsphenamine, 0.9 gram, followed three hours later by a spinal puncture. The spinal fluid at this time showed 240 cells, a four-plus globulin, a positive Wassermann reaction with 0.8 cc. of fluid in the water-bath and a colloidal mastic curve which read 2321000000.

September 7. Neoarsphenamine, 0.9 gram.

September 8. Intraspinal injection of 50 cc. of arsphenaminized serum. The spinal fluid at this time showed 18 cells, a one-plus globulin, was positive with only 1 cc. in the water-bath and with 0.2 cc. in the ice-box. The colloidal mastic curve read 3210000000.

September 10 and 14. Neoarsphenamine, 0.9 gram.

September 15. Intraspinous injection of 20 cc. of arsphenaminized serum.

September 21 to October 19. Five weekly injections of arsphenamine of 0.3 gram each.

Sixty grains of potassium iodide were given daily during his stay in the Hospital. Upon discharge from the hospital the facial paralysis, deafness and vertigo had almost disappeared. Shortly thereafter the patient left the city.

At the time of the first puncture, 3 cc. of the patient's spinal fluid were inoculated into the right testicle of rabbit 177. Thirty-three days later a small nodule could be palpated in the right testicle. Aspiration was not attempted at this time for fear of influencing the course of the lesion. Sixty-eight days after inoculation the nodule was about 2 mm. in diameter, but aspiration

and study by dark field failed to reveal any treponemes. During the next fourteen days the nodule increased rapidly in size until it occupied practically the whole of the upper pole of the right testicle. Aspiration and study of the material of the nodule at this time, eighty-two days after inoculation, revealed many characteristic actively motile Treponemata pallida. The course of the disease in this animal was as follows: Seven days after the organisms were first demonstrated in the right testicle, and eighty-nine days after inoculation a metastatic lesion developed in the left epididymis. Twenty-four days later a bilateral keratitis was noticed. The keratitis has recurred twice since then at intervals of about two weeks. This strain of treponeme is now in the course of further study.

SUMMARY

If the patient's story can be relied upon, treatment in this case was begun while he was still in the seronegative primary stage of the disease and approximately fourteen days after the appearance of the primary lesion. At no time were there any of the usual secondary manifestations. Four months after the last treatment the symptoms of an acute syphilitic meningitis first appeared and one month later the patient applied for treatment. At this time 3 cc. of his spinal fluid, which was positive to all tests, was inoculated into a rabbit's testicle. About sixty-eight days later the animal developed an orchitis and fourteen days later, or eighty-two days after inoculation, many characteristic specimens of T. pallidum were demonstrated in the affected testicle.

REFERENCES

(1) Nichols, H., and Hough, W. H.: Demonstration of Spirochaeta pallida in the cerebrospinal fluid. *Jour. Amer. Med. Assn.*, 1913 (January 11), lx, 108.

(2) Reasoner, M. A.: Early death from cerebral syphilis with successful rabbit inoculation. *Jour. Amer. Med. Assn.*, 1916 (June 17), lxvi, 1917.

(3) Wile, U. J.: The spirochetal content of the spinal fluid of tabes, general paralysis and cerebrospinal syphilis. *Amer. Jour. Syph.*, 1917 (January), i, 84.

(4) Joerss, W.: Spirochätenbefunden im Liquor bei Lues. *Dermat. Wchnschr.*, 1920, lxxi, 966.

(5) Uhlenhuth, P., und Mulzer, P.: Weitere Mitteilungen uber die Infektiosität des Blutes und anderer Körperflüssigkeiten syphilitischer Menschen für das Kaninchen. *Centralbl. f. Bakt.*, 1913, lvii, 158.

(6) Nonne: Syphilis und Nervensystem, 1915.

(7) Ivers, W.: Spirochätenbefunden im Liquor bei Lues. *Dermat. Wchnschr.*, 1920, lxxi, 48.

Hyperplasia of Lipoid-Holding Cells in Diabetes with Lipemia

By Margaret G. Smith

From the Department of Pathology and Bacteriology, The Johns Hopkins University, Baltimore

In rare cases of diabetes with lipemia there has been noted a peculiar change in the spleen, namely, the presence of large numbers of lipoid-holding cells. In these cases the splenic pulp is filled with large, foamy looking cells which contain a lipoid not readily stained by the usual methods. Only four times has this interesting finding been reported. The one now presented is the fifth case.

One such case was reported by Schultze in 1912, 2 by Lutz in 1914, and 1 by Williams and Dresbach in 1917. In these cases slightly different staining reactions for these cells are reported. In Schultze's case only the spleen was involved. With Sudan III, Scharlach R, Nile blue and osmic acid the large cells stained very faintly. By the Smith-Dietrich method with Weigert's iron-hematoxylin and with the myelin-sheath method they stained distinctly. No anisotropic lipoids were seen.

In the first of Lutz's cases the peculiar cells stained in a variety of ways. He divided them into three groups. The first group stained faintly red with Sudan III, a pale rose with Nile blue sulphate, but did not stain with osmic acid. The second group stained red with Sudan III, deep blue with Nile blue sulphate, and not at all with osmic acid. The third group stained with osmic acid, red with Sudan, and bright rose with Nile blue sulphate. All three groups stained with Weigert's iron-hematoxylin. A few anisotropic crystals were present. The reactions for cholesterin were negative. In this case there were a few large cells containing neutral fats in the sinuses of the lymph glands.

The second of Lutz's cases showed a still different staining reaction. With Sudan III a few of the large cells stained bright red, the others a yellow red with Sudan and violet with Nile blue sulphate. All of the cells stained with osmic acid. The reactions for cholesterin were negative. Lutz believed this second case to represent an earlier stage of the process, in which the cells contained mostly neutral fats, and that in the first case the neutral fats had undergone some further change.

In the fourth case, reported by Williams and Bresbach, the large lipoid-holding cells were found not only in the spleen but in the liver and lymph glands. The cells are all said to have stained readily with

Sudan III and Nile blue sulphate, showing considerable amounts of neutral fats and lipoids. Anisotropic lipoids were seen in the spleen and in the Kupffer cells of the liver. The accompanying table summarizes the staining reactions in all these cases.

Family history. Of no importance.

Personal history. The patient's general health had always been good. In August, 1919, he had a gonorrheal infection and at the same time a small sore appeared on the glans penis.

Present illness. Two years previously, in 1917, the patient had an attack similar to that which led to the first admission to the hospital. It lasted two weeks and was

TABLE I

	SUDAN		NILE BLUE SULPHATE	SCHARLACH		OSMIC ACID	MARCHI SOLUTION	SMITH-DIETRICH	IRON HEMATOXYLIN	MYELIN METHOD	ANISOTROPIC CRYSTALS
	I	II After mordanting in potassium bichromate		I	II After mordanting in potassium bichromate						
I. Schultze	Faint		Faint rose	Faint		Faint		+	+	+	−
II. Lutz 1	Faint		Faint rose			−					A few crystals
II. Lutz 2	+		Blue			−					
II. Lutz 3	+		Bright rose			+					
III. Lutz 1	+		Rose			+					
III. Lutz 2	Yellow red		Rose			+					
IV. Williams and Dresbach	+		+								+
V. Author's case 1	+	+	Blue	+	+	+	+	+	+		−
V. Author's case 2	Faint	+	Faint rose	Faint	+	Gray reticulation	0	+	+		
I. Knox, Wahl and Schmeisser 1	+		Violet	+		Faint gray					+
I. Knox, Wahl and Schmeisser 2	Faint		Violet	Faint		Faint gray					
II. Knox, Wahl and Schmeisser 1	+		Violet	+		Faint gray					+
II. Knox, Wahl and Schmeisser 2	Faint		Violet	Faint		Faint gray					
III. Siegmund			Faint rose	Faint	+			+			−
IV. Fahn and Stamm	−		−								+

The following is a report of a similar case.

CLINICAL HISTORY

The patient, a young white man of twenty-two years, was first admitted to the Johns Hopkins Hospital on November 27, 1919. The complaint was "constipation and belching of gas."

characterized by constipation, flatulence, and a sour taste in the mouth. There was no vomiting nor jaundice. Beginning November 2, 1919, he had a gastro-intestinal upset. There was a dull epigastric pain with tenderness in this region, a sour taste in the mouth, and vomiting on one occasion. The symptoms continued with increasing severity until the first admission, November 27, 1919. There was obstinate

constipation. The stools were small and light gray. The appetite was good. There had been a loss of about 20 pounds in weight since the onset of the present illness. There had been dizziness, shortness of breath, nycturia, polyuria, and extreme thirst.

Physical examination on first admission. The patient was under-nourished and a little drowsy. There was a slight fullness in the epigastrium, and the liver edge was palpable. There was a gonococcal urethritis. Blood examination: R.B.C. 4,800,000; Hb. 95 per cent; W.B.C. 10,800; differential count, normal; Wassermann reaction negative. Blood CO_2 combining power, 24.3 volumes per cent. Blood sugar, 0.292. Urine: Specific gravity, 1.028; sugar, 4+; acetone, present.

Course in hospital. The patient improved steadily and was discharged on December 30, 1919, with the urine sugar-free on a 1500-calorie carbohydrate-free diet.

Course after discharge. The patient returned weekly to the dispensary until February, 1920, when he felt so well that the visits were discontinued. The diet was not rigidly kept from that time. On May 1 he developed a perineal abscess which was opened and drained for three weeks. At that time, it is said, the urine showed from 1.5 to 10 per cent sugar. He suffered from constipation, distension and abdominal distress and frequently felt drowsy. From June 30 he continued to grow rapidly worse and had been very drowsy and thirsty until he went into coma on the morning of July 7.

Second admission. The patient was in deep coma from which he never recovered. The blood sugar varied from 0.444 to 0.5, the blood CO_2 combining power from 12.8 to 15 volumes per cent. During the last twenty-four hours there were numerous involuntary stools, all tarry black and strongly positive for hemoglobin. The patient died about thirty hours after he was brought into the hospital. The autopsy was performed two hours after death.

Anatomical diagnosis. Acute pancreatitis; peripancreatic fat necrosis; lipemia; lipoid cell hyperplasia of spleen; splenic infarcts; glycogen infiltration of kidney tubules; diphtheritic ileocolitis; lobular pneumonia.

AUTOPSY PROTOCOL (IN BRIEF)

The body is that of a well nourished young white man. There are deep scars of old perineal abscesses. When the first incision is made, the peculiar creamy appearance of the blood is very striking. Smears stained with Sudan III show many fat droplets. There are no blood-clots in the heart or blood vessels.

Heart. Weight 390 grams. Normal.

Aorta. There are slight atheromatous patches at the root of the aorta.

Lungs. Weight of left lung, 260 grams. Weight of right lung, 380 grams. The bronchi contain aspirated stomach contents. In the upper and middle lobes of the right lung are areas of pneumonic consolidation.

Spleen. Weight, 420 grams. Measurements, 9.5 x 6 x 3 cm. The spleen is quite firm. On the surface there are four small, pale, hard areas which are apparently infarcts. The remainder of the spleen has a deep red color. The splenic pulp shrinks slightly below the cut edge of the capsule. The fibrous tissue trabeculae show distinctly. The Malpighian bodies are plainly seen.

Pancreas. Weight, 130 grams. The head and part of the body form a large, firm, grayish-white mass. All about it are patches of white opaque fat necrosis. The tail of the pancreas is apparently normal. The duct opens into the ampulla close to the common bile-duct. There is no obstruction of the main pancreatic duct; it can be traced through the hard mass of tissue at the head into the normal-appearing part of the pancreas. On section through the head of the gland it shows lobules of rather hard, yellowish, glandular tissue separated by bands of tissue having the opaque white appearance of fat necrosis. Sections through the tail of the pancreas show normal-appearing pancreatic tissue. There are extensive areas of fat necrosis in the subperitoneal fat about the pancreas.

Stomach and duodenum. The stomach is greatly dilated and filled with dark greenish fluid. The gastric and duodenal mucosa is very hyperemic.

Liver. Weight, 2340 grams. Normal. The common duct is patent and there is no evidence of gall-stones.

Adrenals. Weight of both together, 20 grams. Normal.

Kidneys, bladder and ureters. Normal.

Intestines. The mucosa of the small intestines is hyperemic but otherwise normal until within 50 cm. of the ileocecal valve. Beginning here and extending through the large intestine, the mucosa is very rough and is covered with a thick diphtheritic membrane. When the intestinal wall is cut through, many punctate hemorrhages are seen in the submucosa. In some places the thick diphtheritic membrane has been rubbed off, leaving a very red and granular surface.

Bone-marrow. Not obtained.

MICROSCOPIC FINDINGS

Heart muscle. Normal with hematoxylin and eosin stain.

Lungs. There are areas of fresh lobular pneumonia.

Adrenal. Normal.

Kidney. The tubular epithelium is swollen. Many tubules contain granular debris. Parts of the convoluted tubules have large, clear, epithelial cells probably containing glycogen, but stains were not made to demonstrate this.

Liver. With a hematoxylin and eosin stain the liver tissue appears normal. There are small fat globules in many cells, especially those about the portal areas, which stain red with Sudan III and blue with Nile blue sulphate. The Kupffer cells show nothing unusual with a haematoxylin and eosin stain. With a Sudan III stain on formalin material a few small droplets of well stained fat are occasionally seen in Kupffer cells as well as in the liver cells. There are no large cells resembling those in the spleen.

Pancreas. Sections from the tail show well preserved pancreatic tissue. There is little or no increase of connective tissue. The acinic cells take a rather bright eosin stain. The islands in some sections are few and small, but in others are quite plentiful and of fair size. There are a few areas of small-round-cell infiltration in one section, but no other evidence of an inflammatory process. In sections from the head, however, there is an entirely different picture. Here the lobules are separated by an increased amount of interstitial tissue and fat which is partially necrotic and infiltrated with polymorphonuclear leukocytes and large mononuclear phagocytic cells. The ducts are better preserved than the surrounding tissue, giving the impression that this was not primarily an ascending infection of the pancreatic ducts. Often the inflammatory process invades the lobules of pancreatic tissue and produces faintly stained, partially necrotic acini. The islands are numerous and where not in the midst of necrotic tissue are well preserved and not scarred. A few large Gram-positive cocci are scattered through the necrotic part of the tissue.

Lymph glands. There is an increase in the number of phagocytic cells in the sinuses of the mesenteric lymph glands. With a hematoxylin and eosin stain many of these cells are clearly seen to be vacuolated. Some are round, others are spindle-shaped; but they do not at all resemble the large, round, foamy cells of the splenic pulp. With a Sudan III stain these cells contain large droplets of fat which stain deep red. In the small capillaries of the glands there are droplets of fat, sometimes free and sometimes apparently in the cells of the lining endothelium.

Spleen. The microscopic appearance of the spleen, when stained with haematoxylin and eosin, is identical with that in the other cases reported. The pulp is filled with closely packed, large, round or oval cells, which stain a pale bluish-pink. The sinuses, on the other hand, are unusually distinct and practically free from these cells. Only after a careful search is an occasional one found free in a sinus. The endothelial cells of the sinuses are not swollen and seem entirely normal. The interesting cells which fill the pulp have a finely vacuolated, foamy protoplasm which suggests that some substance has been dissolved during preparation of the section. The nuclei are usually small and stain deeply with hematoxylin.

Fat stains were made on this tissue after four years' fixation in formalin. With Sudan III and Scharlach R some of the endothelial cells of the sinuses show small, deeply stained fat droplets. Droplets of readily stained fat are also found free in

the sinuses and small blood vessels as well as within polymorphonuclear leukocytes.

Only a few of the peculiar large cells in the pulp contain droplets of fat which takes a bright red stain with Sudan III and Scharlach R, and these brightly stained cells are slightly larger than the remaining cells. The remaining large cells stain only very faintly with Sudan III and Scharlach R. This is due to the fact that they contain only a small amount of stainable fat distributed in minute droplets, so that with the low power the stain appears diffuse and faint. With the high power these tiny droplets, although distinctly seen, show only a pale, yellowish-red stain. A few of the cells take none of the Sudan III or Scharlach R stain.

With osmic acid practically all of the cells stain to some extent. A few, the slightly larger cells, are filled with large black droplets. Others contain smaller droplets of varying size. But most of them are stained only a faint gray and under the high power show, not droplets of fat, but a peculiar gray reticulation. This appearance is evidently caused by the reaction of the osmic acid only on the periphery of each fat globule. Some of the cells stain with Marchi fluid, showing droplets of fat. Most of these are apparently the same cells which show the large droplets with osmic acid, Sudan III and Scharlach R. The remaining cells are not stained by the Marchi method.

Again, with Nile blue sulphate the few larger cells show droplets stained deep blue while the remaining cells take only a very faint diffuse pink tinge, or no stain at all. With a polarizing microscope no anisotropic fats are seen.

With Sudan III, after mordanting the sections in potassium bichromate solution, the few slightly larger cells show large droplets stained deep brownish red, while every cell shows very finely divided fat stained orange red. With iron hematoxylin, after mordanting in potassium bichromate solution, all these cells stain a deep slate blue. By the Smith-Dietrich method all the cells stain a pale slate blue.

In summary, then, part of the lipoid material usually contained in cells which are slightly larger than the others, is unsaturated fatty acid, for it stains blue with Nile blue sulphate and still stains with osmic acid when the Marchi method is used. But there is present additional lipoid which does not react as fatty acid, pure glycerin or cholesterin esters, or cholesterin. It stains by the Smith-Dietrich method, and evidently belongs to the class of difficultly stainable lipoids for which no method of further differentiation has been devised.

Four other extremely interesting cases of an apparently similar nature, but not associated with an evident lipemia, have been reported. Two of these cases were reported by Knox, Wahl and Schmeisser, one by Siegmund, and one by Farr and Stamm. These cases have all occurred in infancy, the oldest patient dying at four years, the youngest at nine months of age. All of these children were girls. Two of them were sisters and of a Hebrew family. The race of the other two is not reported.

Case I (4). The patient was nine months old when first seen. She had been breast fed for three months and then fed on cow's milk mixtures. There had been frequent attacks of vomiting until six months of age. The gain in weight had been very slow. At nine months she weighed only 11 pounds and in the next two months before her death her weight fell to 8½ pounds. No record of the urine examination is given in the report. The blood examination showed on admission: R.B.C. 5,200,000; W.B.C. 10,400; Hb. 90 per cent; differential count: P.M.N. 42 per cent, L.M. 5 per cent, S.M. 41 per cent. Later the blood examination showed R.B.C. 4,400,000; W.B.C. 35,000; Hb. 60 per cent; differential count: S.L. 82 per cent, L.L. 9 per cent, P.M.N. 3 per cent. The Wassermann reaction was negative. In the physical examination the large size of the liver and spleen were the striking findings. The child was very pale and showed a slight brownish pigmentation of the skin.

Case II (4). The second patient, a sister of Case I, was first seen at five months of

age when she weighed 9 pounds. She had been exclusively breast fed. She had always been thin and sickly, although she had never had an acute illness. She was pale and had a grayish-yellow color. Three months later she was admitted to the Johns Hopkins Hospital. She was difficult to feed satisfactorily. She vomited frequently, had attacks of diarrhea and constipation and gained little in weight during her stay in the hospital. The abdomen was frequently much distended. Furuncles and eczema were often present. She had otitis media at one time. The physical examination showed a large spleen and liver as in Case I. No sugar or albumin were found in the urine in the three examinations reported in the hospital records. The blood examination showed on admission: R.B.C. 3,024,000; W.B.C. 11,240; Hb. 65 per cent; differential count; P.M.N. 34 per cent, P.M.E. 1 per cent, P.M.B. 1.5 per cent, L. 60 per cent, transitionals 3.5 per cent. Later it showed R.B.C. 4,608,000; W.B.C. 6,480; differential count; P.M.N. 37 per cent, L.M. 19 per cent, S.M. 39 per cent, Transitionals 1 per cent, Myelocytes 3 per cent. The Wassermann reaction was negative. An interesting ophthalmological finding is reported. There was a blurring of the discs and a cherry-red spot in each macula. There were no other evidences of amaurotic family idiocy except the increasing emaciation. The child died at fifteen months of age of exhaustion and emaciation.

At autopsy the findings in these 2 cases were much alike. The striking finding was the presence of large numbers of lipoid-holding cells in many organs—in the liver, spleen, adrenal, renal glomeruli, lymph glands, thymus and lymphatic tissue of the intestinal tract in the first case. In the second case, in addition to these organs the bone-marrow and even the brain tissue is said to have been implicated. The staining reaction of these cells, as shown in the table, is practically the same as in the lipemia cases. There was a considerable amount of doubly refractile lipoids. In both cases the cells in the spleen uniformly filled the spaces between the distinctly outlined sinuses.

Case III (5). The patient was a nine-month-old child whose sister is said to have died during the first year and to have had a large spleen and liver. This child also showed a very large spleen and liver. No sugar or albumin was found in the urine. The child died of an intercurrent pneumonia. The hyperplasia of lipoid-holding cells was present in the spleen, liver, bone-marrow and lymph glands and renal glomeruli. In the description of the spleen, the pulp and reticulum cells are said to be changed into large vacuolated cells while the endothelium of the sinuses is not implicated in the process. The cells stained palely in formalin-fixed material with Nile blue sulphate, and with Scharlach R, but in tissue fixed in potassium bichromate they took a bright yellow-red stain. They stained with the Ciaccio and Smith-Dietrich methods, with Spielmeyer's myelin sheath method and with Weigert's iron-haematoxylin. There were no doubly refractile fats.

Case IV (6). The fourth case, a child of four years, had diabetes as well as a nephritis, but the diabetes was of a very mild character. The sugar in the urine disappeared during the last few months of life and the blood sugar was increased slightly (0.101 to 0.117) and that only early in the period of observation. Death was believed to be due to nephritis rather than to diabetes. The child was poorly nourished and had developed slowly. She weighed only 8.5 kgm. at three years of age and gained only 0.75 kgm. during the next year. The blood examination showed: R. B. C. 3,920,000; W.B.C. 10,400; Hb. 77 per cent. The Wassermann reaction was negative. In this case the spleen was only moderately enlarged, measuring 8 cm. in length. The liver is not said to have been at all enlarged. The same large cell hyperplasia was present in the spleen, lymph glands and liver. The distribution of these cells in the spleen is not discussed. The lipoid did not stain with Sudan III or with Nile blue sulphate. Much doubly refractile lipoid was present.

There is a striking similarity in the first three of these cases in age, in

the clinical history and findings, and in the pathological anatomy. In these three cases the lesion is said not to be localized in the reticulo-endothelial system. Large lipoid-holding cells are described in the renal glomeruli, and in the second case even the brain tissue contained large vacuolated cells. The question should be raised whether these large cells found outside the reticulo-endothelial system really are of the same nature as the others. In the second of these cases the change in the brain closely resembles that found in amaurotic family idiocy and, considering the ophthalmological findings during life, it may well be that such a condition was present in this case and that the large cells in the brain are changed brain cells rather than phagocytic cells like those in the spleen. The staining reactions of these cells are not mentioned. It is possible that the few lipoid-holding cells in the renal glomeruli, which stained with Sudan in at least the first two cases, are only cells of the glomerular tuft which contain fat droplets and are not similar to the phagocytic lipoid-holding cells of the reticulo-endothelial system; or they may be phagocytic cells carried there by the blood stream. At least careful study and consideration should be given such cases before this change is accepted as occurring in cells outside the reticulo-endothelial system.

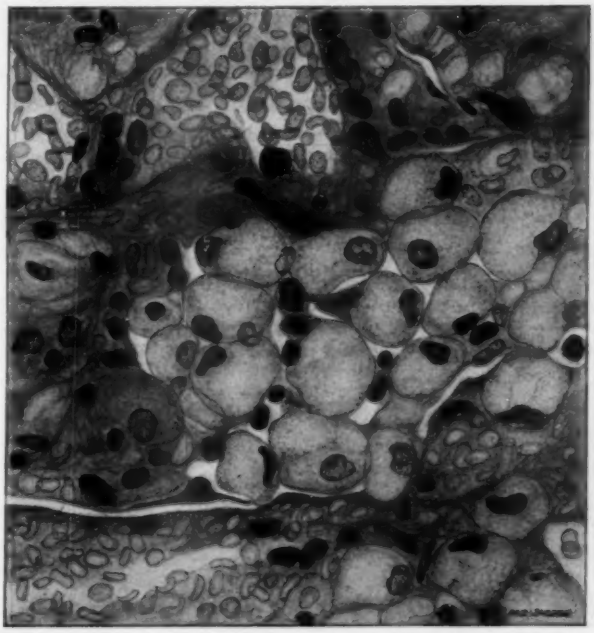


FIG. 1. SHOWS A SPLENIC SINUS AT THE TOP AND ANOTHER AT THE BOTTOM OF THE FIELD WITH THE PULP SPACE BETWEEN FILLED WITH THE LARGE LIPOID-HOLDING CELLS

In all four of these cases the staining reactions of the characteristic cells are similar to each other and practically the same as in the cases occurring with diabetic lipemia. The cells in the spleen are arranged uniformly throughout the pulp with normal sinuses between, just as in the cases of diabetic lipemia. Mandelbaum and Downey, discussing the first two of these cases, have pointed out that the peculiar cells differ from those occurring in Gaucher's disease not only in this uniform arrangement in the spleen but also in their staining properties and in their morphological appearance. In a recent case of Gaucher's disease seen in this laboratory the microscopic pathology is entirely different from that of these cases and of the cases associated with lipemia in diabetes. The fourth case, occurring in a child, differs clinically from the other three and resembles the case here reported in the fact that the patient had had diabetes. But the diabetes was only in a very mild form. There was no lipemia visible at autopsy.

There is again a resemblance between all of these cases with peculiar lipoid-containing cells and the lipoid cell hyperplasia found in experimentally produced cholesterinemia, but in this condition the lipoid-holding cells contain large amounts of doubly refractile lipoids, and the lipoid substances stain readily with Sudan III. The endothelium of the sinuses is also changed and these swollen endothelial cells, as well as the large cells in the pulp, are filled with a lipoid which is readily stained with Sudan III.

Although the extent of involvement varies and the staining reactions differ somewhat, yet the resemblance in all of these cases is striking enough to suggest that the underlying conditions may be closely akin. Some disturbance of lipoid metabolism is probably present in all of them. The fact that the lipoids differ in the different cases, and even in different cells in the same case, suggests either that the substance taken up by the cells has undergone some change within the cells or that an unusual excess of more than one lipoid is circulating in the blood and is being phagocytized by these cells.

SUMMARY

A case of diabetes with lipemia, in which there was a peculiar hyperplasia of lipoid-containing cells in the spleen, is reported. Four other cases having the same change associated with lipemia in diabetes have been collected from the literature. These 5 cases are compared with 4 unusual cases of a similar hyperplasia of lipoid-containing cells occurring in small children; 1, associated with a very mild diabetes without a recognized lipemia, and 3 without any evidences of diabetes.

REFERENCES

(1) Schultze, W. H.: Ueber grosszellige Hyperplasie der Milz bei Lipoidaemie (Lipoidzellenhyperplasie). Verhandl. d. d. pathol. Gesellsch., 1912, xv, 47.

(2) Lutz, Wilhelm: Ueber grosszellige Hyperplasie der Milz pulpa bei diabetischer Lipämie. Ziegler's Beitr., 1914, lviii, 273.

(3) Williams, John R. and Bresbach, M.: A fatal case of diabetes mellitus associated with large-cell hyperplasia. Amer. Jour. Med. Sci., 1917, cliii, 65.

(4) Knox, J. H. Mason, Jr., Wahl, H. Boswell, and Schmeisser, H. C.: Gaucher's disease; a report of two cases in infants. Johns Hopkins Hosp. Bull., xxvii, i, 1916.

(5) Siegmund: Lipoidzellenhyperplasie der Milz und Splenomegalie Gaucher. Verhandl. d .d. pathol. Gesellsch., 1921, xviii, 59, 1921.

(6) Fahr und Stamm: Zur Frage d. sog. Lipoidzellen-Hyperplasie. Klin. Wchnschr., 1924, iii, 1206.

(7) Mandelbaum, F. L., and Downey, H.: The cases of Gaucher's disease reported by Drs. Knox, Wahl and Schmeisser. Johns Hopkins Hosp. Bull., 1916, xxvii, 109.

(8) Anitschkow, N.: Ueber experimentell erzeugte Ablagerungen von anisotropen Lipoidsubstanzen in der Milz und im Knochenmark. Ziegler's Beitr., 1914, lvii, 201.

The Fate of the Sutures as Observed in Intestinal Anastomosis and in the Healing of Wounds[1]

By Frederick Leet Reichert and Emile Holman

From the Hunterian Laboratory of Experimental Surgery, Johns Hopkins Medical School

THE purpose of this report is to present certain observations prompted by the stimulating discussions of the late Professor Halsted during the course of his last experimental study on intestinal suture.[2] In his article, which may be read and re-read with never failing interest and profit, he says concerning this subject;

> Who knows, for example, how much of the intestinal wall should be turned in; whether two rows of stitching are better than one; whether the suture should be continuous or interrupted; whether the Lembert or mattress stitch is preferable; if the knots should be on the mucous or on the peritoneal surface; why some stitch-loops (knot outside) fall into the lumen, and others remain on the peritoneal surface; who has considered the factors facilitating or delaying the release of the inturn; and who, indeed, has endeavored to estimate the weight of the burden thrown upon the experimentee to counteract the operator's shortcomings?

In the same article Professor Halsted gave a description of an aseptic end-to-end anastomosis in which the blind ends of the divided bowel are approximated with a single row of interrupted mattress sutures, the lumen being reëstablished by means of a sharp knife introduced *per rectum*.

Our observations were in part made on a series of 38 animal experiments performed by Doctor Halsted, in which this type of anastomosis was employed. Detailed notes were made at the time of the operation recording the amount of trauma and possible soiling, the type and number of stitches used, the side on which the knots were situated, the estimated size of the flange or inturn, and whether or not the operator believed that the stitches had entered the lumen. At necropsy, careful observations on the fresh specimen were made as to the presence and extent of adhesions, the number and exact location of stitches, the degree of unfolding and size of the flange, and the presence or absence of dilatation of the bowel. The specimens were then cleared by the Spalteholtz method, as described by Sabin,[3]

[1] Received for publication, January 25, 1925.

[2] Halsted, W. S.: Blind-end circular suture of the intestine. Closed ends abutted and the double diaphragm punctured with a knife introduced *per rectum*. *Ann. Surg.*, March, 1922, pp. 356–364.

[3] Sabin, F. R.: On the fate of the posterior cardinal veins and their relation to the development of the vena cava and azygos in the embryo pig. *Contributions to Embryology*, 1915, vol. 3, Carnegie Institute. Washington, Publ. No. 223.

which rendered them quite transparent, and enabled us to determine the exact fate of the silk suture, its migrations through the intestinal wall, and the unfolding of the inturned flange. Observations made by this method were so striking that further experimental studies were undertaken to determine the fate of sutures in the different types of intestinal anastomosis in common use. These led also to a careful study of the process of repair following the suture of abdominal wounds.

INTESTINAL ANASTOMOSIS

A uniform procedure, within the limits of the operators, was carried out in the 38 experiments in which Doctor Halsted's blind-end circular method was used. Of the experiments, twenty-one were performed by Doctor Halsted, and the remaining operations were done by members of his staff. Nine of the specimens were obtained within ten days after operation to observe the early processes of healing; the remaining twenty-nine specimens were obtained at various times from 10 to 153 days after operation.

Slight trauma and soiling were recorded at the initial operation in 13 cases. Eight of these 6, 7, 9, 15, 16, 53, 64 and 80-day specimens revealed firm fibrous adhesions about the site of the anastomosis, and only five had moderate omental adhesions. In the cases uncomplicated by trauma or soiling there were no adhesions whatever in five of the 58, 68, 91, 109 and 153-day specimens, and only slight filmy adhesions in nineteen ranging from 24 to 88 days. Adhesions were, however, invariably noted in all the early specimens, and it was apparent that these always occur but gradually disappear if not too extensive or too fibrous.

Again, in the absence of trauma and soiling, it was noted that the inturned bowel or flange had entirely straightened out in five of the 22, 63, 71, 109, and 153-day specimens, was only slightly present in seventeen, and moderately present in seven. All of these last seven specimens were obtained within four days after the operation. In cases complicated by trauma or soiling, the flange invariably persisted longer, being absolutely unchanged in one 112-day specimen, moderate in seven of 6, 7, 9, 53, 54, 80 and 89 days, slight in four of 15, 16, 83 and 97 days and absent only in one of 87 days.

The ultimate fate of the silk sutures employed provided a fascinating study. Cleared specimens in 27 cases revealed a slowly progressive migration of the great majority of the mattress sutures so as to lie free on the surface of the bowel covered only by peritoneum. For example, in five, 26, 63, 71, 76 and 89-day specimens, respectively, all the stitches were present and accounted for, and lying free, underneath the serosa (Fig. 1, No. 34). The older specimens show a beginning absorption of this silk as it lies underneath the peritoneum (Fig. 1, No. 10) without macroscopic reaction about it, and with only a few lymphocytes observable on microscopical examination. In fifteen other specimens, one or several sutures were found hanging in the lumen of the bowel, attached only by a small bit of tissue, the knot hanging free. What factors, then, determine whether a stitch will migrate

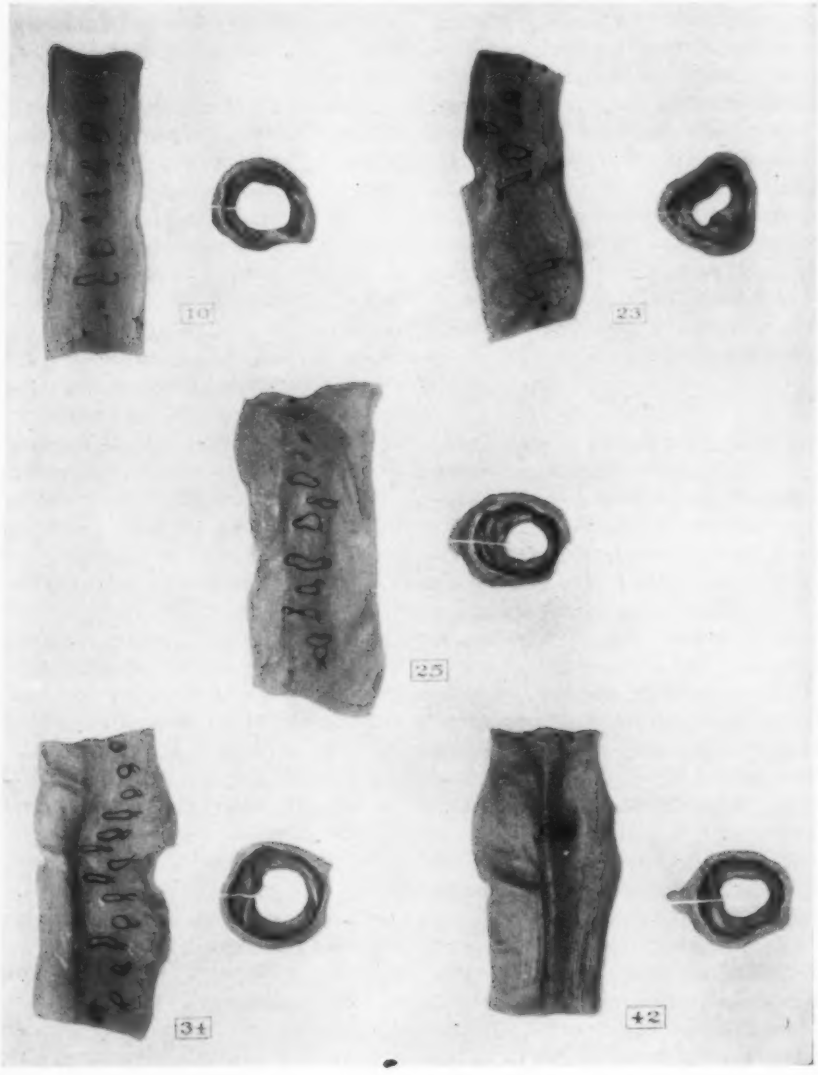


FIG. 1. SEGMENT OF BOWEL, OPEN AND CLOSED, CONTAINING LINE OF END-TO-END ANASTOMOSIS OF COLON—ASEPTIC METHOD OF DR. HALSTED

See bottom page 215 for explanation of Fig. 1

into the lumen or to the peritoneal surface? A possible explanation was revealed in the following experiments:

Five end-to-end anastomoses were performed according to Doctor Halsted's aseptic method. Mattress stitches were used throughout, but in each instance an effort was made to pass the needle through the full thickness of the bowel wall and to make it enter the lumen. From this group, two of the animals developed peritonitis within five days; the remaining three were sacrificed on the 29th, 34th and 42nd days. In the latter specimens there were firm adhesions around the site of union, and a moderate flange was still present. Peritoneal adhesions had not formed in the two cases in which peritonitis had ensued and leakage was present around the stitches, undoubtedly accounting for the infection. In the 29-day specimen, seven of the original stitches were hanging in the lumen, and two were found to have migrated toward the peritoneal surface of the bowel wall. A small abscess had formed between one of the latter stitches and the omentum. In the 34-day specimen, all of the thirteen stitches were present, and all of them were hanging within the lumen. Always the knot lay free in the lumen, although it had originally been tied on the peritoneal surface of the bowel. The findings were precisely the same in the 42-day specimen (Fig. 2, No. 12).

In a second group of four anastomoses, also performed according to the aseptic method, the stitches were applied with the greatest care in the hope that the lumen would not be entered. The specimens were obtained on the 5th, 6th, 28th and 100th days. No adhesions were present in the 100-day specimen and the inturned flange had disappeared. Eleven of the original thirteen stitches were found; four apparently had migrated toward the lumen, the other seven away toward the serosa. There were slight adhesions around the line of union in the 28-day specimen and only two of the stitches showed signs of migrating toward the lumen. The inturned flange had almost disappeared. In the five and six-day specimens there was no evidence that the

All views are from the serosa.

All specimens in this and succeeding figures have been cleared, and the illustrations were made from them by Mr. William B. McNett.

10: 100 days: All silk mattress sutures lie beneath the serosa, some partially absorbed.

23: 91 days: No silk within lumen. Seven of the original ten mattress, two Lembert stitches and four blood vessel ligatures have worked outwards and lie just beneath the peritoneum.

25: 89 days: At operation a hematoma formed where a crushing clamp had been applied, and at this point very little of the flange has unfolded, and the two sutures remain deep. All of the original remaining thirteen sutures are present; they have lodged just beneath the serosa.

34: 71 days: All of the original thirteen mattress and one Lembert suture are beneath the serosa; ten of these lie entirely on the side of the knot.

42: 53 days: Trauma and slight soiling at the time of operation. Of the original thirteen mattress and two Lembert sutures only one mattress and several blood vessel ligatures remain. The others migrated into the lumen. The procedure was new to the operator and presumably the stitches entered the mucosa.

stitches were migrating toward the lumen. In the 6-day specimen the inturned flange had already unfolded halfway.

The factors responsible for the migration of the suture, whether inwardly into the lumen, or outwardly so as to lie on the peritoneal surface of the bowel, appear to center around the knot. It can be seen from the cleared specimens that most of the sutures which have not penetrated the lumen

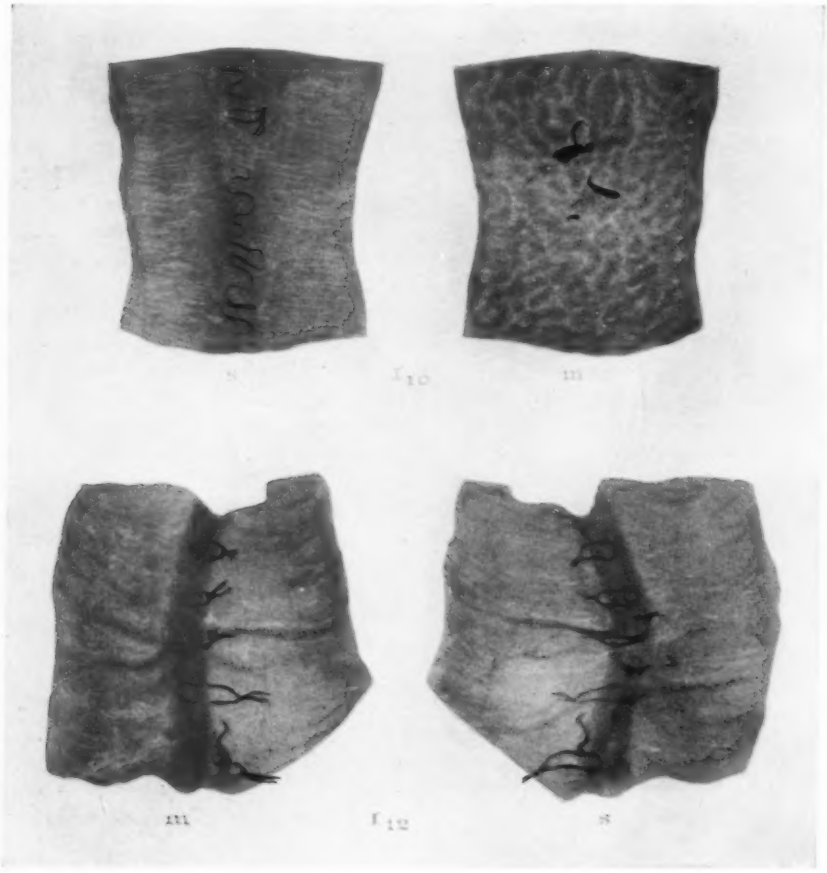


FIG. 2. SAME ANASTOMOSIS OF COLON

10: 100 days: Continuous Connell silk suture. Most of the stitch lies beneath the serosa (*s*) but both ends of the knot are dangling from the mucosa (*m*).

12: 42 days: Lumen deliberately entered with five mattress sutures; all are hanging in the lumen and in each the knot is free (*m*).

(*s*) View from the serosal surface showing sutures in the depth.

finally come to lie free on the peritoneal surface of the bowel, having pulled completely across the line of union to the side on which the knot originally lay. To accomplish this, the included muscle and submucous fibers must have been divided, presumably by pressure necrosis, the division probably proceeding simultaneously with the unfolding of the flange. On the other hand, when a suture pierces the lumen, the infection which follows apparently initiates an entirely different sequence of events, for the knot, although originally placed on the peritoneal surface, is then invariably found in the lumen, and eventually the suture is extruded into the lumen, and lost.

Somewhat similar observations were made on an 89-day specimen (Fig. 1, No. 25). In the operative note the statement was made that a slight hematoma had occurred in the suture line, as a result of the application of a clamp. When the specimen was cleared (Fig. 1, No. 25), all of the original thirteen mattress stitches were accounted for. Eleven were lying free on the peritoneal surface of the bowel covered by peritoneum. At the site of the hematoma the two mattress sutures lay more deeply buried. At this point the flange was quite wide, whereas along the remainder of the line of union it had almost entirely disappeared. This specimen provided a good object lesson in the harmful effects of hemorrhage and trauma upon subsequent healing, and also upon the unfolding of the flange.

In another, a 53-day specimen (Fig. 1, No. 42), the anastomosis had been performed by one unacquainted with the method, and as a result considerable trauma had been inflicted and apparently all of the stitches had penetrated the lumen. At any rate, on clearing, only one mattress stitch was recovered; presumably all the others had dropped off into the lumen. There were firm omental adhesions around the site of anastomosis and the flange had not unfolded half-way.

One 13-day and one 23-day specimen were obtained following end-to-end anastomoses with the interrupted Connell sutures, tied inside the bowel. All these sutures were found hanging in the lumen, none having migrated toward the peritoneal surface. In one specimen, three mattress sutures had been applied to close the last bit of opening in the bowel. These three stitches appeared on the peritoneal surface, whereas the Connell sutures all remained within the lumen. There was a moderate flange in each case with good healing of the mucosa.

A very interesting 100-day specimen (Fig. 2, No. 10) was obtained following an anastomosis with the use of a continuous Connell suture applied through all the coats of the bowel. There were firm omental adhesions but no flange; excellent healing of the mucosa had occurred. The two terminal knots were hanging in the lumen, but the intervening portion of the stitch lay on the peritoneal surface of the bowel covered only by peritoneum. A non-absorbable suture of this kind might be a source of danger, inasmuch as an inflammatory reaction with deposition of excessive fibrous tissue might conceivably follow an infection traveling along the suture line.

Interrupted Lembert sutures were employed in one operation, but the animal died on the following day.

Death was due to a general peritonitis, following leakage along the line of union and through the stitch holes. The use of Lembert sutures alone in intestinal anastomosis has long been considered a dangerous procedure and this case appears to corroborate that impression.

In an endeavor to make deductions as to the comparative merits of lateral anastomosis as performed (1) by the

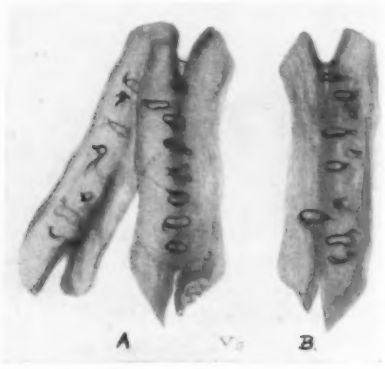


FIG. 3. LATERAL ANASTOMOSIS OF SMALL INTESTINE

28 days: Presection mattress sutures. (A) posterior view: the mattress sutures have been migrating to the serosa; (B) anterior view: most of the sutures have been deposited on the side of the knot.

method originally described by Professor Halsted in 1887, in which only one row of interrupted sutures of the mattress type were utilized both posteriorly and anteriorly, and (2) by the method most generally employed, in which two rows of continuous sutures of catgut were used, a number of experiments were performed by Professor Halsted and by Dr. Karl Schlaepfer of his staff. The anastomoses were made without the use of the intestinal clamps. The six specimens from animals operated upon by Dr. Halsted were obtained from two to five weeks after operation; three showed moderate omental adhesions at the site of the anastomosis, and three revealed only slight adhesions. There was evidence of a slight inflammatory reaction in two cases, but limited to a few of the silk knots. The flange was still present in all cases, but had diminished about half in width in the older, five-week specimens. With one exception all the black silk mattress sutures, which had been originally applied so as to avoid entering the lumen, were lying subperitoneally, or had migrated in that direction. Only one suture was hanging in the lumen with the knot end free. Even though in these experiments the knots in the posterior line of suture had been buried from view at the original operation, the cleared specimens showed all stages of migration of these sutures to their final resting place immediately under the peritoneum (Fig. 3).

In the animals operated upon by Dr. Schlaepfer, the specimens were obtained in 3 to 21 days after operation. All showed moderate adhesions. Three showed no evidence of infection, whereas in the remaining three there was definite inflammatory reaction around the line of union. The width of flange and its persistence varied considerably, being most conspicuous in the areas where unusual trauma had been inflicted, as by tying knots. In certain areas the flange had completely disappeared as early as two weeks after operation. In his experiments green-colored catgut (No. 0 plain for the inner row, No. 0 chromic

for the outer row) welded, as it were, on needles, was used, and as early as the third day the inner row had become interrupted at numerous points, presumably by digestive processes. At the same time the outer row of catgut could not be identified in two specimens, and in a third was partially absorbed. On the fourteenth and twentieth days there was no evidence of any residual catgut whatsoever. Both types of anastomosis were apparently equally effective, and healed correspondingly well.

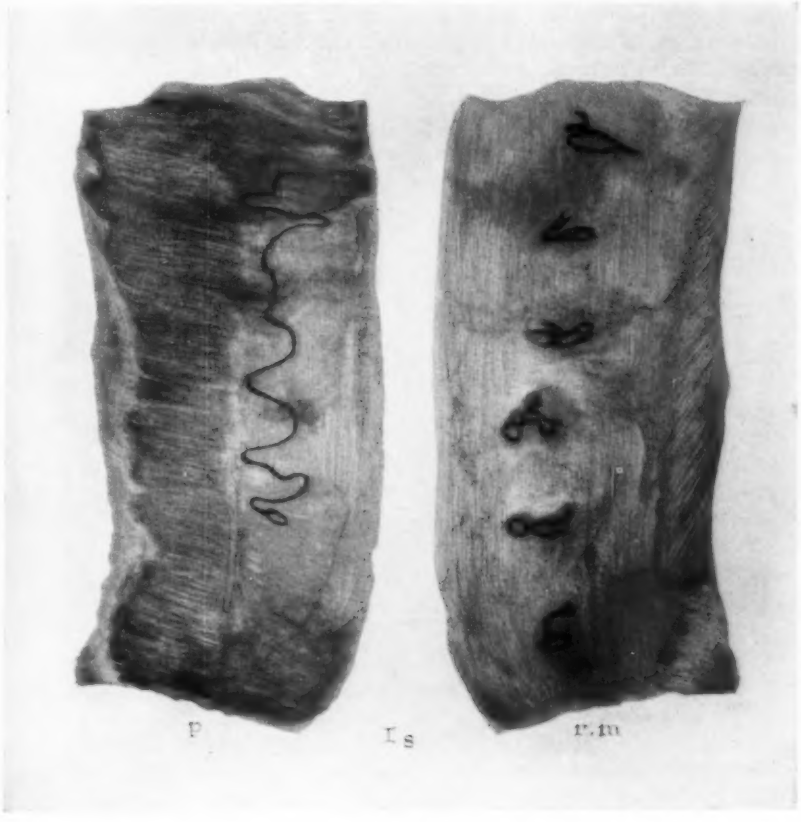


FIG. 4. CLOSURE OF ABDOMINAL WALL. INTERRUPTED DOUBLE SILK SUTURES

100 days: Specimen consisting of rectus sheath, muscle and peritoneum has been cleared. *p* = peritoneal view; the continuous silk peritoneal stitch is covered by endothelial cells. *r.m.* = anterior view. The lower three stitches were tied tightly and have cut through and are lying on the sheath, whereas the upper three were tied loosely and the rectus and anterior sheath are intact.

Abdominal wall closures. In approximating the peritoneum and

posterior sheath, either a single continuous "whip" suture was used, or the edges were everted and united by a continuous Cushing stitch. Healing occurred in all cases without underlying peritoneal adhesions, and within two weeks the silk thread could be seen from the peritoneal side, covered by a thin layer of endothelium. In closing the anterior sheath, no attempt was made to include any muscle tissue. The edges were approximated with interrupted stitches of fine silk doubled in one of three ways: (*a*) by a simple single suture; (*b*) by a horizontal figure-of-eight suture; (*c*) by a vertical figure-of-eight suture (Fig. 5). The simple sutures were tied either to approximate the edges loosely, or to constrict the included tissue tightly. It was found that the former sutures remained accurately in place and caused only a slight tear in the sheath (upper three sutures Fig. 4) with an occasional untying of the suture. Invariably, however, the sutures which had been tightly tied caused large defects in the sheath, and were frequently found lying quite free on the surface of the sheath (lower three sutures Fig. 4), particularly in the older specimens.

FIG. 5. CLOSURE OF ABDOMINAL WALL. FIGURE-OF-EIGHT SUTURES

100 days: *p* = peritoneal view. The partially digested continuous peritoneal suture is covered with endothelium.

r.m. = anterior view: The changed position of the seven loosely tied figure-of-eight sutures is shown. In several the small loop of the stitch has been released, preventing tension necrosis of the anterior sheath.

Similar effects were produced by the horizontal and vertical figure-of-eight sutures, depending upon the degree of tension to which the included tissue was subjected. It was apparent in each instance, as shown by the fresh and cleared specimens, that good repair and healing were best obtained when loosely tied sutures were applied, and that the tight suture was more harmful and injurious than effective (Figs. 4 and 5). It is of interest to note that in the figure-of-eight suture it was the small loop that invariably cut through the tissues first, thus relieving tension and preventing any further tearing of the fascia by the larger loop.

SUMMARY

1. Silk sutures employed in intestinal anastomosis migrate either to the peritoneal surface and are covered only by a transparent layer of endothelial cells, or to the lumen of the bowel, into which they eventually drop off and are lost. When the sutures do not enter the mucosa they migrate toward the peritoneum. If the mucosa is pierced they slough into the lumen. The presence or absence of infection along the stitch determines in which direction the sutures will travel.

The fate of various sutures is described.

Absorbable material would seem to be superior when through-and-through sutures are required.

2. The flange of inturned bowel soon disappears when trauma or soiling does not complicate; but persists, with long delayed fibrous healing when either or both of these factors are added.

3. The degree of omental adhesions at the site of anastomosis is also dependent upon trauma and soiling.

4. No difference was noted in the healing about a lateral anastomosis when a single row of interrupted mattress silk sutures or a double row of continuous catgut sutures was used.

5. Sutures used in the closure of abdominal wounds, when applied so as to constrict the tissues which are included, invariably produce defects in the anterior sheath. Sutures applied so loosely as just to approximate the edges of the wound did not injure the tissues. The vertical figure-of-eight suture is better than the horizontal figure-of-eight. Simple vertical sutures are as efficient as the vertical figure-of-eight suture.

6. The results emphasize the importance of gentleness and care in handling tissues.

Book Reviews

The Biology of Death. By RAYMOND PEARL, Ph.D. $2.50. (*J. B. Lippincott Company, Philadelphia, 1924.*)

Written in the author's usual vivid and cogent style, there is not a dull moment during the perusal of this little book. The thoughtful student may well afford to spend several delightful hours with it; in fact he can ill afford to miss it.

The problem is thus stated:

"1. Why do living things die? What is the meaning of death in the general philosophy of biology?

"2. Why do living things die *when* they do? What factors determine the duration of life in general and in particular, and what is the relative influence of each of these factors in producing the observed result?"

In the elucidation of the problem the author has brought together "under a unified viewpoint some of the more important contributions which have been made to our knowledge of natural death, from three widely scattered sources: namely, general biology, experimental biology, and statistical and actuarial science." He brings out speedily the fact that death is not an inherent consequence of life and cites the potential immortality of the protozoa, of the germ cells of the metazoa and even of the somatic cells of higher forms of life when these cells are removed from the body and placed in a suitable environment. The somata of multicellular organisms die because of the differentiations and specializations of structure and function which have been developed at the expense of the power of their cells to maintain an independent and indefinitely continued existence.

There are entertaining chapters on the conditions of cellular immortality, the chances of death, the causes of death on an organological basis and on the basis of the author's interesting embryological classification. The inheritance of duration of life in man is convincingly set forth in one chapter followed by one on experimental studies on the duration of life. As the keynote of the whole we may quote "the strong chain of evidence which indicates the highly important part played by innate constitutional biological factors as contrasted with environmental factors in the determination of the observed rates of human mortality."

Medical thought seems now, perhaps, to be tending toward a more definite interest in the pathology of constitution and, it is to be hoped, to a more adequate study of that important subject. There is the interesting possibility that medicine may take cognizance of current fundamental biological research, especially that relating to the study of heredity and of development. If so, the student can find no more attractive introduction to the idea than by reading "The Biology of Death."

F. P. S.

Differential Diagnosis. By RICHARD C. CABOT, M.D. Volume 2. Third Edition. 709 pages. $9.00. (*W. B. Saunders Company, Philadelphia.*)

In this third edition the introductory sections have been elaborated and changed somewhat but the form and the case histories are the same.

Diagnostic ability rests not alone on oft repeated observation of clinical syndromes, but also upon the study of the complete course of disease until terminated by cure or by death. Apparently incontrovertible conclusions derived from the collation of symptoms, of signs and of data from the laboratory, must be tried by the ultimate information gained from surgical exploration or from dissection of the cadaver. Opportunity to control clinical impression and so to test the accuracy of reasoning at the bed-side is not available to all. This lack has been supplied, in a measure at least, by the mode of presentation adopted by the author in this work.

The nineteen subjects presented are dis-

cussed in a practical way that stresses the salient points of each. A prefatory general discussion of each symptom includes a consideration of its causes, the conditions in which it appears, the frequency of its occurrence and of its corroborative signs. This is followed by a detailed recital of illustrative cases. The facts needed to establish a differential diagnosis are then set forth, the interpretation of the author is given and the validity or the error of the conclusion is discussed in the light of information gleaned from the outcome of the case or from data obtained in the operating room or at the post-mortem table.

The method of presentation adopted by the author is well adapted to emphasize the problems of diagnosis and to show how often conclusions must be corrected when inference is controlled by fact.

Lucidly written, in an easy style, this work serves further to show how honesty with one's self is a *sine qua non* for the development of diagnostic skill.

A more complete index would enhance the usefulness of this really excellent book.

H. T.

Diseases of Middle Life. The prevention, recognition and treatment of the morbid processes of special significance in this critical life period, comprising twenty-two original articles by various eminent authorities. Edited by Frank A. Craig, M.D. In two volumes. $15.00. (*F. A. Davis Company, Philadelphia, 1923.*)

In this work of two volumes twenty-three different authors have contributed articles on the important diseases of middle life. It shares with other systems the advantages and disadvantages of this method of presentation. It differs from most other systems of medicine chiefly in the facts (1) that fewer subjects are considered, (2) that these, as a rule, are more fully presented and (3) that sections on gynecology, diseases of the male genitourinary tract, diseases of the skin and diseases of the eye are included. There are also articles dealing with the general subjects of diet, exercise and occupation.

On the title page, in the preface and in the introduction, emphasis is placed upon the importance of the prevention of the maladies that have the most important bearing upon the length of life of the individual and upon the efficiency and well-being of old age. Accordingly, one expects to see rather more attention than usual paid to prophylaxis and to the detection of early evidences of disease. This expectation is, however, not realized. In the article of 207 pages on diseases of the heart, six and a half are devoted to the prevention of those diseases, whereas of the 107 pages allotted to the subject of diabetes, one has sufficed for the discussion of prophylaxis. In most of the articles the idea of prevention receives no special notice.

The list of contributors contains many well known names and most of the articles have been handled very satisfactorily.

W. T. L.

The National Health Series. Edited by the National Health Council. $6.00 per set.—20 volumes in the set. (*Funk & Wagnalls, New York City.*)

The National Health Series edited by the National Health Council, and written by foremost leaders in their line, contains the last word in almost any subject relating to public health in an authoritative manner. In the volume, "The Child in School—Care of Its Health," by Thomas D. Wood, M.D., while the purpose of health supervision and inspection of school children is simply defined as (1) the discovery of defects and disease; informing the parents about this, and persuading the parents to obtain or accept advisable attention, we find the 77 pages of this little book crowded with information on every angle. This takes from "What the Child of School Age Ought to Be" to "What the Citizen Ought to Do" to accomplish it.

Another volume is on "Home Care of the Sick" by Clara D. Noyes, R.N. Miss Noyes recognizes that most nursing of our land is done in the home. She has provided for mothers a book written particularly for them. Miss Noyes' rich experience in this subject through her connection with the program of instruction of the American Red Cross in home hygiene and care of the sick throughout the land, makes her especially qualified to write on it.

Another volume, "Exercises for Health," by Lenna L. Meanes, M.D., Medical Director, Women's Foundation for Health, Inc., is intended particularly for the middle-aged women of the nation who most need scientific medical education. Space does not permit more than mentioning the other volumes here, of equally high order, which are a part of this remarkable series: "Health of the Worker—How to Safeguard it," by Lee K. Frankel, Ph.D.; "Your Mind and You—Mental Health," by George K. Pratt, M.D., and "Adolescence—Educational and Hygienic Problems," by Maurice A. Bigelow, Ph.D.

J. B. M.

Smallpox and Vaccination. By BENJAMIN WHITE, PH.D. $1.00. (*Harvard University Press, Cambridge.*)

Here is a clear, succinct presentation of the vaccination problem as it exists today. In this small book, the thirteenth in a series of Harvard Health Talks, after a sketchy survey of the clinical and historical aspects of smallpox, Dr. White emphasizes the rôle of vaccination in combating the disease and warns against the wiles of the anti-vaccinationists. He reviews briefly the history, technique, and efficacy of vaccination, and supports the claims by statistics comparing the incidence of smallpox in states having compulsory and in those having only voluntary vaccination. He presents the subject in a form sufficiently popular to appeal to the laity and yet in a manner sufficiently scientific to warrant the attention of medical men. From the strictly medical view-point it is inadequate, although it is a timely rebuttal to the campaign arguments brought forward by the anti-vaccinationists. It should certainly be in the hand of every legislator who must face their propaganda.

W. B. S.

Anesthesia for Nurses. By WILLIAM WEBSTER. $2.00. (*C. W. Mosby Company, 1924.*)

This small volume is not intended to be a textbook in anesthesia for nurse-anesthetists, as might be inferred from its title, but is written for the surgical nurse with a view to increasing her understanding of anesthesia. For this purpose, however, the book seems too long and technical. Little of its text could be applied to the nurse's daily work, and the chapters which would be most instructive to her—those about pre-operative and post-operative care, and the point-of-view of the patient—are dealt with somewhat briefly.

Nurses in country districts, who are called upon to give anesthetics without instruction and experience, would learn more by studying a simple description of one or two methods of anesthesia that could be applied to any type of case, with a graphic recital of signs and complications, such as is found in Dr. J. G. Poe's paper on Modern General Anesthesia, read before the State Medical Association of Texas, May 12, 1921.

Students in anesthesia will derive the greatest benefit from reading this volume before attempting to study the larger textbooks, such as that of Flagg and that of Gwathmy. Every word of the text is borne out by experience. However, to the trained anesthetist it seems little more than a brief reiteration of all that is contained in all modern books on anesthesia.

Two facts noted in the text stimulate comment. An explanation for the remarkably good results obtained with chloroform for maternity cases, given by anyone available, is based on the fact that these mothers-to-be have no fear of the drug and welcome the relief from pain which it brings. Does this not suggest the importance of gaining the confidence of our patients and of overcoming apprehension in order that the course of the anesthetic be smooth and the after-effects uncomplicated?

On page 47 we read the following sentence: "The cornea is often insensitive to touch." There are so many ways of drawing deductions as to the depth of anesthesia, that this method seems quite unnecessary and decidedly risky.

In conclusion, it may be said that the student in anesthesia who wishes a concise survey of the subject in its entirety could do no better than to read this book.

M. B.